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THE AMERICAN REVIEW OF TUBERCULOSIS

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MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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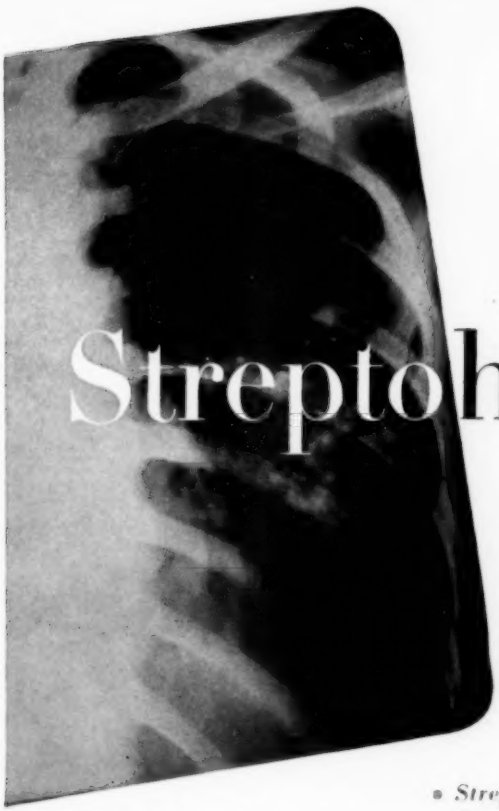

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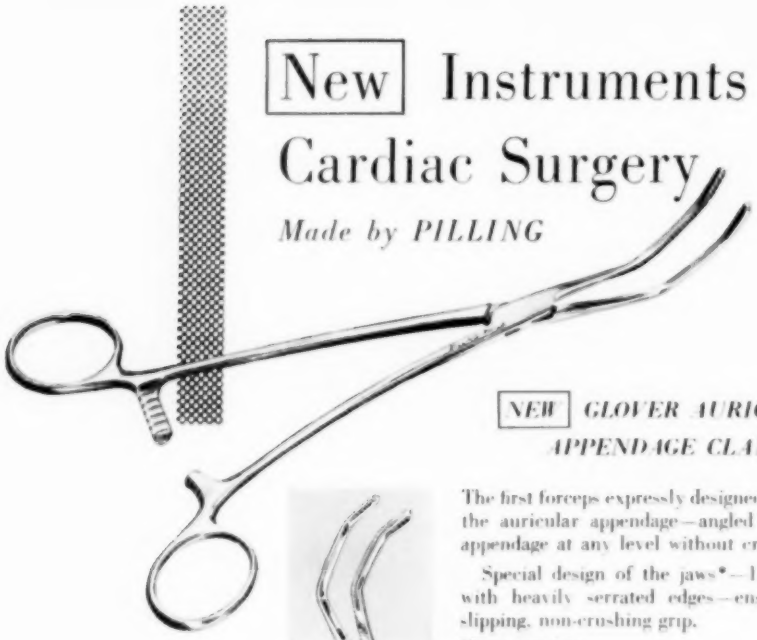

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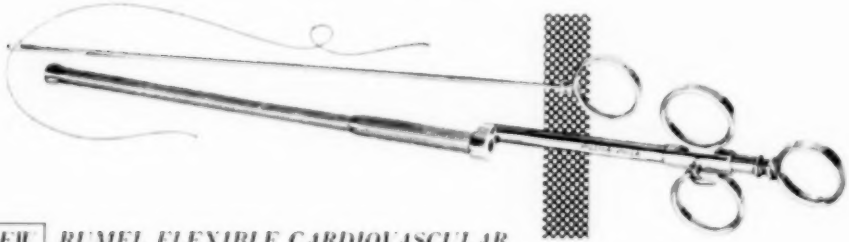

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THE AMERICAN REVIEW
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VOLUME 67 *June, 1953* NUMBER 6

TUBERCULOSIS TODAY AND TOMORROW[1]

DAVID T. SMITH

(Received for publication January 5, 1953)

INTRODUCTION

The rapidly declining death rate from tuberculosis in the United States, Canada, and Western Europe has led many persons, including budget committees of federal and state governments, to reach the false conclusion that tuberculosis is under control and will be eliminated in the near future. Figure 1 shows that the steady decline which has been going on for decades has acquired a more precipitous descent since 1947.

The slow decline is the result of many factors, including (*1*) improvement in the standard of living, (*2*) treatment in sanatoriums, (*3*) earlier diagnoses, and (*4*) reduction in the incidence of infection by detection and isolation of the open carrier cases. The decline has been very slight in Spain and Portugal, and the death rate has actually increased in some parts of Asia. These are areas where the standard of living has remained stationary or has declined in recent years. There also are the areas without good case-finding programs and adequate sanatorium beds. The precipitous drop in the death rate since 1947 corresponds chronologically with the introduction of streptomycin and para-aminosalicylic acid (PAS) and the perfection of the surgical technique for pulmonary resections.

It is significant that the decline has been equally as dramatic in Iceland, New Jersey, Minnesota, and Iowa (where BCG vaccination has not been employed) as in Norway, Sweden, and Denmark, where a considerable proportion of the population has been vaccinated with this immunizing agent.

It is the opinion of Drolet and Lowell (*5*) that the sudden decline in the death rate since 1947 is an artifact resulting from improved methods of therapy and does not reflect a corresponding decline in the incidence of the disease. It can be seen in figure 2 that a death rate of 70 in 1930 was accompanied by a new case rate of 100 per 100,000. By 1950 the death rate was only 22 while the new case rate was 80. The death rate in Scotland, shown in figure 3, dropped from 90 in 1930 to 70 in 1938, and the case rate dropped from 200 to 170 during the same period of time. During World War II, however, the death rate returned to 80 and the case rate to 200.

[1] Address presented before the annual meeting of the Minnesota Tuberculosis and Health Association, Minneapolis, Minnesota, October 27, 1952.

The persistent high case rates are to some extent a reflection of improved case-finding methods. This is certainly true in Canada, where a death rate of 79 and a case rate of 64 in 1930 was replaced by a death rate of 46 and a case rate of 117 in 1945. Many cases have been found by mass roentgenographic surveys in recent years, but the statistical studies of Drolet and Lowell (5) indicate that these surveys have not materially inflated the case rates but have detected more persons with curable disease and thus accelerated the declining death rate.

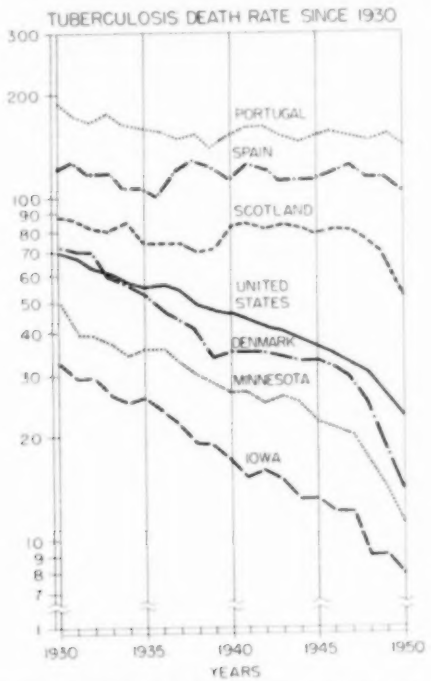

FIG. 1. Death rate per 100,000 population between 1930 and 1950. Note the precipitous decline in all of the curves from the year 1947 except in Portugal and Spain. (Data from study by Drolet and Lowell, Dis. of Chest, 1952, *21*, 527).

The accompanying figures (figures 2 and 3) show conclusively that the death rate from tuberculosis in the United States, Canada, and Western Europe can no longer be used, even as a rough indication of the prevalence and relative importance of tuberculosis.

The annual new case rate is a somewhat more reliable guide; but the figures obtained may reflect the vigor of the case-finding program rather than the prevalence of the disease. In Canada in 1930, less than one new case was reported for each death from tuberculosis. Following a marked improvement in their case-finding program, 2.6 new cases were discovered for each death in the year 1945.

For the United States as a whole, in 1951, 3.8 new cases were found for each death, with a low of 2.4 for Texas and a high of 8.6 for Minnesota (table 1) (4). In five rural counties in North Carolina, with insignificant numbers of Indians and Negroes, the average death rate was 3.2 per 100,000 with 19 new cases discovered for each death (12). No one knows what the ratio of new cases to deaths should be or whether this rate will vary widely from area to area. In 1951, the death rate in North Carolina (15.2) was approximately twice that in Minnesota

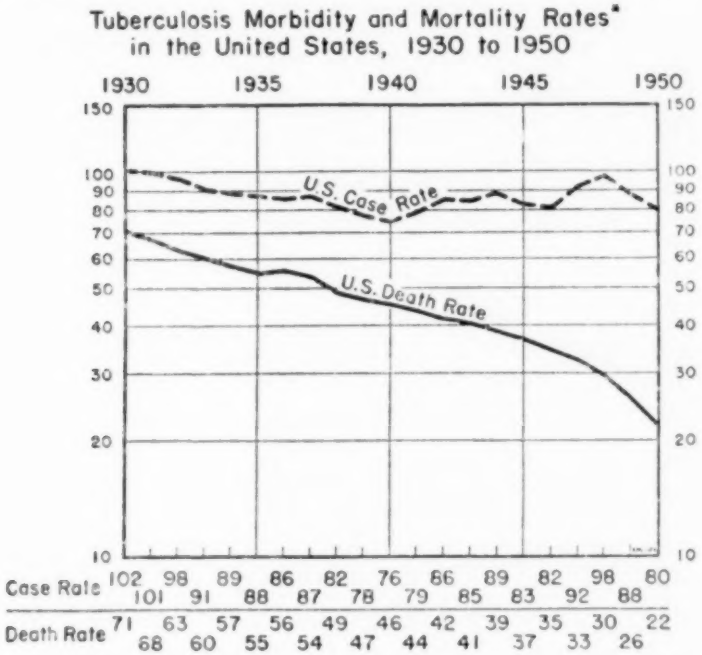

FIG. 2. Morbidity and mortality rates per 100,000 in the United States from 1930 to 1950. The morbidity rate is not declining proportionally to the mortality rate (Drolet and Lowell, Dis. of Chest, 1952, *21*, 527).

(8.5) while the ratio of new cases to deaths in Minnesota (8.6) was exactly twice that in North Carolina (4.3) (4). But in the five counties in North Carolina where the death rate was 3.2 per 100,000, 19 new cases were discovered for each death (12). Does the ratio of 8.6 new cases for each death in Minnesota represent the detection of all the new cases in Minnesota and do the 19 cases for each death in the five North Carolina counties represent a biologic accident;[2] or is Minnesota, with its excellent case-finding program, discovering less than one-half of its new

[2] Mass roentgenographic surveys were conducted for the first time in some of these North Carolina counties in 1950 and 1951.

cases of tuberculosis? It is obvious that some more adequate method of measuring the extent of the tuberculosis problem is necessary.

TUBERCULOSIS AS INFLUENCED BY AGE

Between 1800 and 1900 in Europe and in the Northeastern states of this country, tuberculosis was so prevalent that more than 99 per cent of the popula-

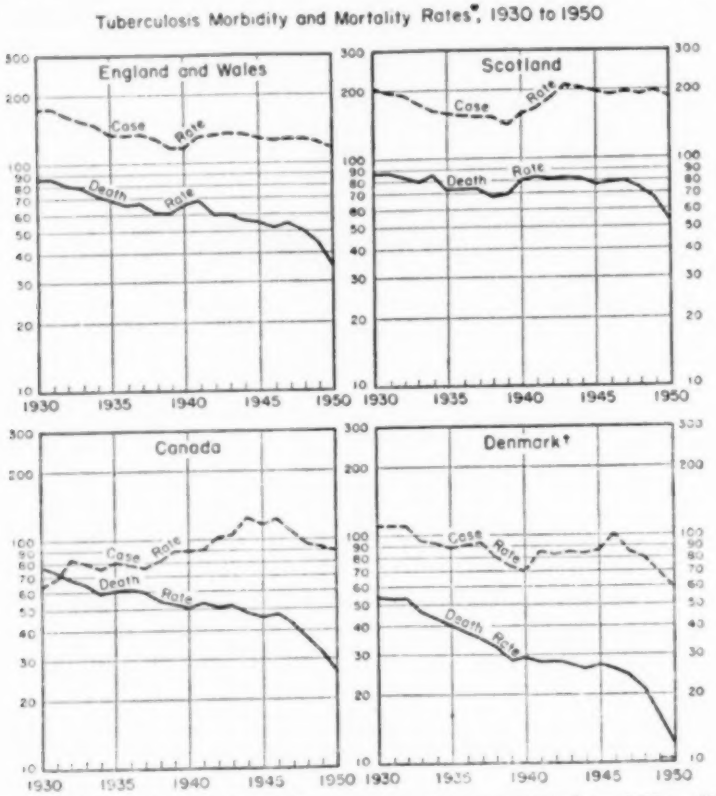

FIG. 3. Tuberculosis morbidity and mortality rates per 100,000 from 1930 to 1950 in England and Wales, Scotland, Canada, and Denmark (Drolet and Lowell, Dis. of Chest, 1952, *21*, 527).

tion had been infected by the age of twenty. Since the population was saturated with tubercle bacilli, the death rate would be a reflection of the genetic resistance of the people as modified by unfavorable environmental factors. The type of age distribution produced by this situation is shown in figure 6 (9). It can be seen that the death rate in infants was as high as 600 per 100,000. This peak was followed by a relatively low rate of 50 to 100 in children between the ages of eight and twelve although the infection rate in these children must have

been even higher than among infants. The sharp rise in the death rate, beginning with puberty, to a peak of 300 per 100,000 at the age of twenty to twenty-five represents the classical reinfection type of tuberculosis found in young adults. There is no reason to suspect that the acquisition of new infections in this period would be appreciably greater than in the eight- to twelve-year period; therefore, we must assume that some rather profound change occurred in the resistance of

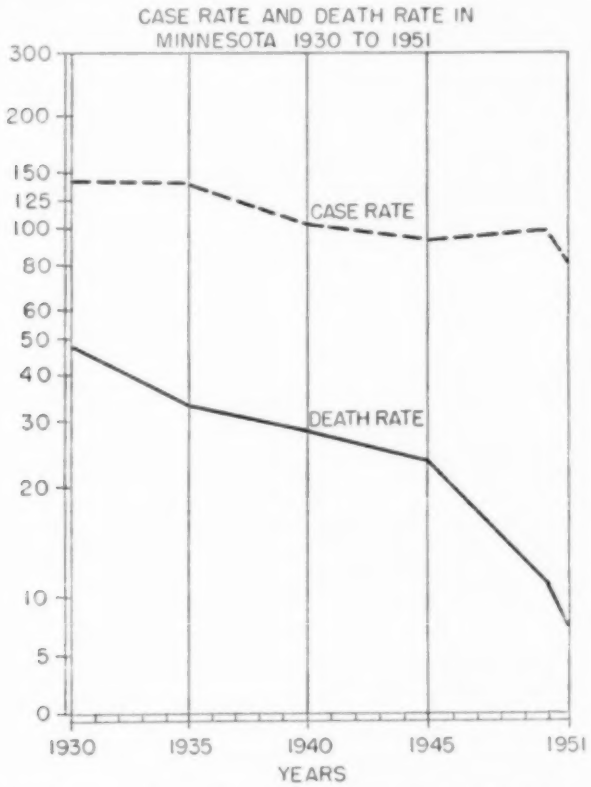

FIG. 4. Morbidity rate and mortality rate in Minnesota from 1930 to 1951. Graph made from data supplied by Dr. D. S. Fleming of Minnesota Department of Health.

these individuals. It has been suspected for many years that this decrease in resistance was brought about by alterations in the endocrine glands which occur at puberty. This theory receives some support from the recent reports of fulminating tuberculosis which developed during therapy with cortisone and corticotropin (1). The rapid decline in the death rate between the ages of fifty-five and seventy-five is a manifestation of the law of the survival of the fittest, since those surviving infection and death in infancy and those surviving the deleteri-

ous effects of the hormone shifts of puberty would be less likely to develop a fatal infection in old age. In general, the curves suggest that the higher the death rate in infancy and in the period of young adult life the lower the death rate will be

FIG. 5. Tuberculosis mortality in children under fifteen years of age in the United States, Minnesota, and Denmark. The decline has been more rapid in Minnesota than in Denmark since 1945 although BCG vaccination has not been used in Minnesota. (Data from study by Drolet and Lowell, Dis. of Chest, 1952, *21*, 527.)

TABLE 1

DEATH RATE AND RATIO OF NEW CASES TO DEATHS, 1951

	DEATHS	RATE	NEW CASES	NEW CASES PER DEATH
United States	30,837	20.1	118,438	3.8
Puerto Rico	2,637	117	6,075	2.3
Arizona	492	64.5	3,772	7.7
Nevada	55	33.8	198	3.6
Minnesota	257	*8.5*	2,208	*8.6*
North Carolina	629	*15.2*	2,724	*4.3*
Five North Carolina counties	9	*3.2*	171	*19*

in old age. This period of old age, however, is not an actual but only a relative period of resistance since the death rate was as high as 25 to 40 per 100,000.

FACTORS INFLUENCING THE DEATH RATE BY AGE

The curves shown in figure 6 may be considered as typical for a population with a relatively good genetic resistance and a relatively good standard of living

when exposed to universal tuberculization. If the standard of living drops precipitously, as it did in some parts of Europe during World War I, all parts of the curve are elevated. When the standard of living is materially improved, all parts of the curve are depressed although the reduction in infant mortality is less striking than that observed in the eight- to twelve-year age period and the twenty- to twenty-five-year period.

If a population with less genetic resistance is exposed to universal tuberculization as were the Negro Senegalese soldiers in France in World War I, the death

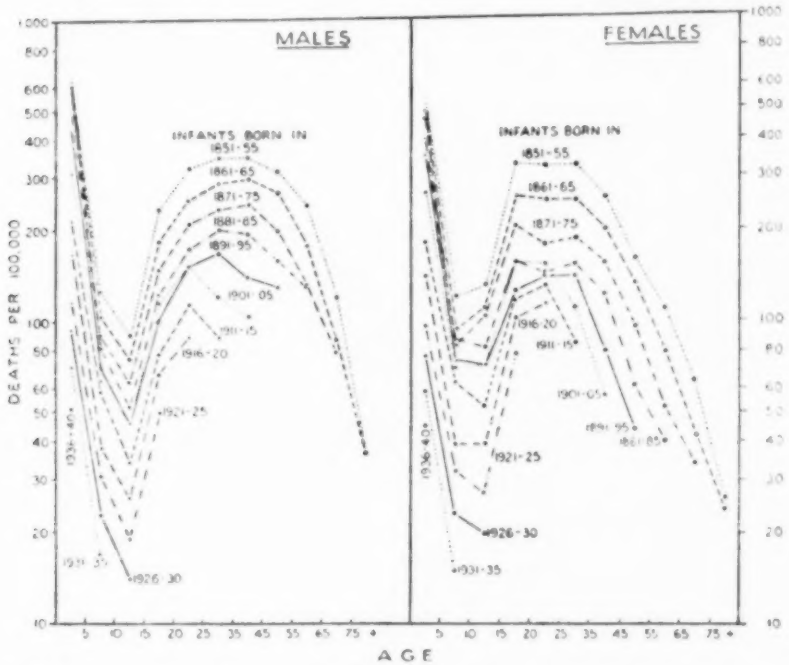

FIG. 6. Mortality from tuberculosis in Sweden of different ages in the same generation (John B. McDougall, *Tuberculosis*, Williams & Wilkins Company, Baltimore, p. 218).

rate in young male adults may reach the appalling figure of 1,114 per 100,000. In contrast, with a population of greater genetic resistance than the Swedes, such as the Jews of Western Europe, the death rate is appreciably lowered even though the environmental factors are not as good.

Dr. J. A. Myers (10, 11) has emphasized the importance of the death rate in infants and children as an index of the degree of tuberculization in the population. With a reduction in mass infection, the death rate in infants and children shows a drop twenty-five years before that of adult life and fifty years before that of the older age groups. Figure 5 shows a slow but steady decline in the death rate in children in the United States between 1930 and 1949. The rather

precipitous drop in Sweden since 1946 has been attributed to the use of the BCG vaccine but the even more dramatic decline in Minnesota has been accomplished without the use of BCG.

Apparently the death rate in infants and children can be used as an index of the reduction in mass infection provided the population under study is emerging

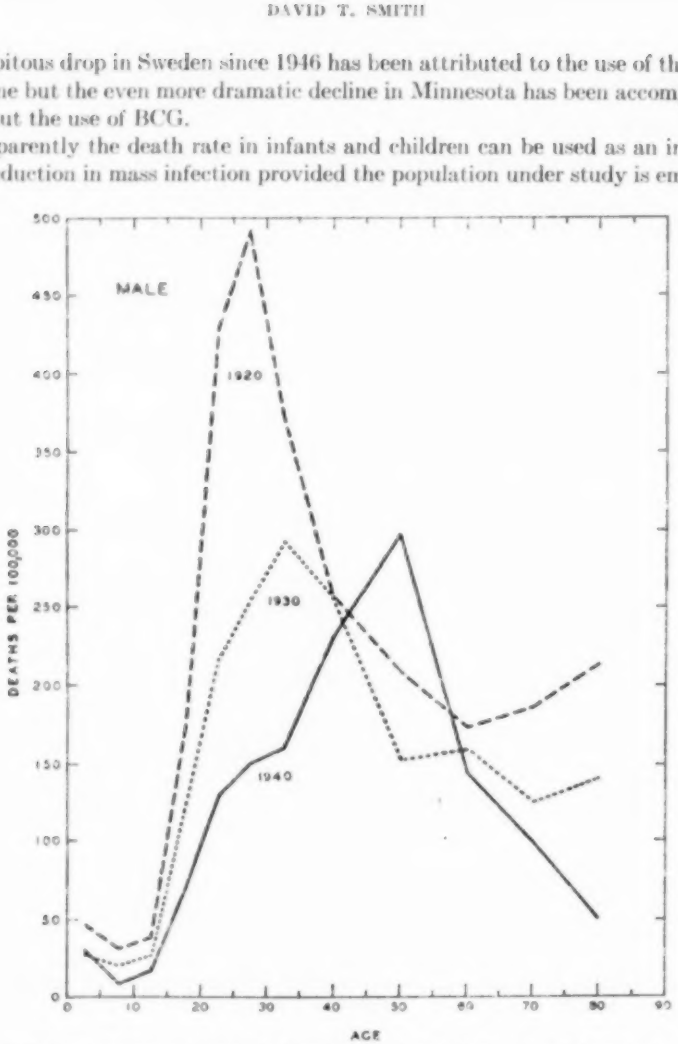

FIG. 7. Mortality rate per 100,000 in Negro males in North Carolina. Note the low death rate in children under twelve years of age (Carroll E. Palmer, U. S. Public Health Service, 1943, vol. 1).

from a period of total tuberculization. It is not a reliable index, however, in a population where an estimated 40 per cent of adults have negative tuberculin reactions such as the Negro males of North Carolina (figure 7).

FACTORS INFLUENCING THE SHAPE OF THE CURVE OF DEATH BY AGES

The degree of tuberculization at different ages alters materially the shape of the tuberculosis curves. If one compares the curves for white males (figure 6) in

Sweden, born between 1851 and 1855, with the Negro males (13) in North Carolina in 1920 (figure 7), it can be seen that infants in Sweden had a death rate of 600 per 100,000 while Negro infants in North Carolina had a death rate of only 50 per 100,000. But males in Sweden had a death rate of 300 per 100,000 at the age of twenty-five while North Carolina Negro males had a death rate of 490

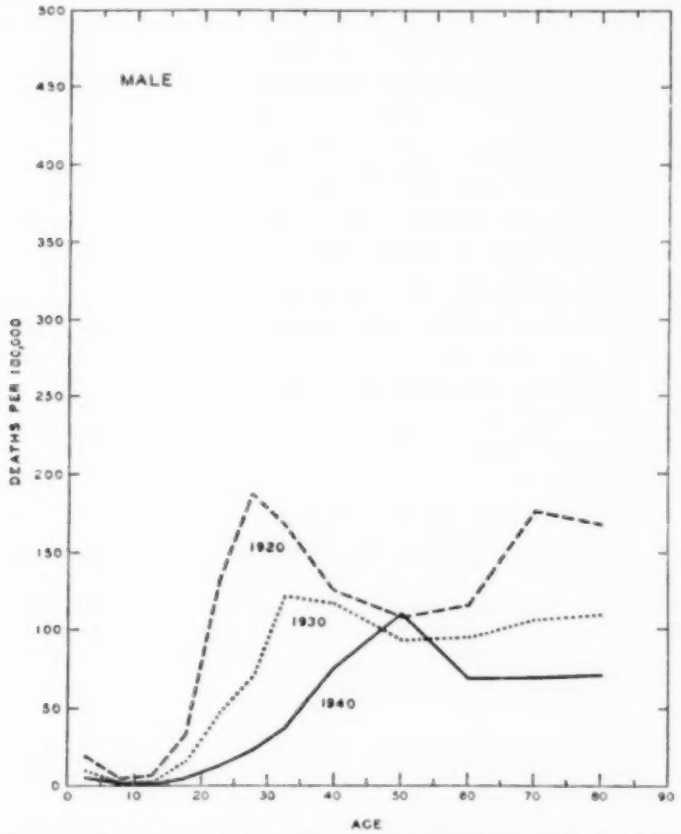

FIG. 8. Mortality rate per 100,000 in white males in North Carolina. Note the low death rate in children under fifteen years of age (Carroll E. Palmer, U. S. Public Health Service, 1943, vol. 1).

per 100,000. Negro infants and children have a relatively low rate of tuberculization (2, 3). In 1937, McCain (8) found that only 18 per cent of the Negro school children gave positive tuberculin reactions. The high peak of 490 in the Negro males at the age of twenty-five years is partly from reinfection tuberculosis and partly from rapidly fatal primary infections.

The height but not the shape of the curve is controlled by the genetic resistance of the Negro. Roth's (14) study of tuberculosis in Negro soldiers and white sol-

diers in the United States Army between World War I and World War II showed that Negro soldiers developed tuberculosis only slightly more frequently than white soldiers but had a death rate nearly four times as high. This was a well-controlled, if accidental, experiment since the food, clothing, housing, and medical care were identical in the two groups. The death rate among Negroes in North Carolina is only three times that in the white population. The infection rate in North Carolina is less in both white and Negro citizens than among the population of the large eastern cities, where the Negro death rate may be five or six times as high as among the white population. Although the cash income of the rural Negro in the South is much less than that of the Negro in the northern cities, the Southern Negro has a better diet, more rest, and is less crowded than the Negro who has migrated to the slums of the northern cities.

A comparison of the death rates for Negro males and white males in North Carolina for 1920, 1930, and 1940 (figures 7 and 8) shows that the maximum death rate for both races has shifted from twenty-five years of age in 1920 to fifty years of age in 1940. Up to the age of fifty in all three periods, the death rate for Negro males was approximately three times that of white males. In 1920 and 1940, the death rate for Negro males between seventy and eighty years of age was materially lower than that of white males of the same age. The higher rate in 1930 may reflect the more severe impact of the 1930 depression on the Negro population. The lower death rate in old age may have resulted from the elimination of many susceptibles by the excessively high death rate between the ages of twenty and fifty.

TUBERCULOSIS IN MINNESOTA AND NORTH CAROLINA

Certain similarities and contrasts are obvious when the tuberculosis problems of Minnesota and North Carolina are compared. Minnesota has a population of approximately 3,000,000 white citizens. The largest city in North Carolina has a population of 200,000, but approximately 1,500,000 people live in cities varying in size from 25,000 to 200,000. Approximately the same number are concentrated in two or three cities in Minnesota. The rural white population of each state is roughly 1,500,000.

There was never very much bovine tuberculosis in the small scattered dairy herds in North Carolina in contrast to the heavy infection in the large Minnesota dairy herds. Tuberculin testing in school children between 1920 and 1930 showed a positive reaction rate of 47 per cent in the school children of Minneapolis (10). Surveys between 1920 and 1924 revealed a rate of 13.9 per cent for the school children of the state with a rural rate in 1924 of 10 per cent (15). Between 1930 and 1933, a majority of the school children of North Carolina were tuberculin tested by the State Board of Health under the direction of the late Dr. P. P. McCain (8). A positive reaction rate of 14 per cent was found for white children and 18 per cent for Negro children. Some rural counties, with few or no Negro students, have a rate as low as 5 per cent.

These figures indicate that there was as much, if not more, tuberculous infection in Minnesota as in North Carolina between 1920 and 1930. A comparison of the death rate in white infants, as seen in figures 8 and 9, shows a rate which

was twice as high in Minnesota as in North Carolina. It is probable that the high incidence of bovine tuberculosis in the Minnesota cattle explains the increased rate in the Minnesota infants (6).

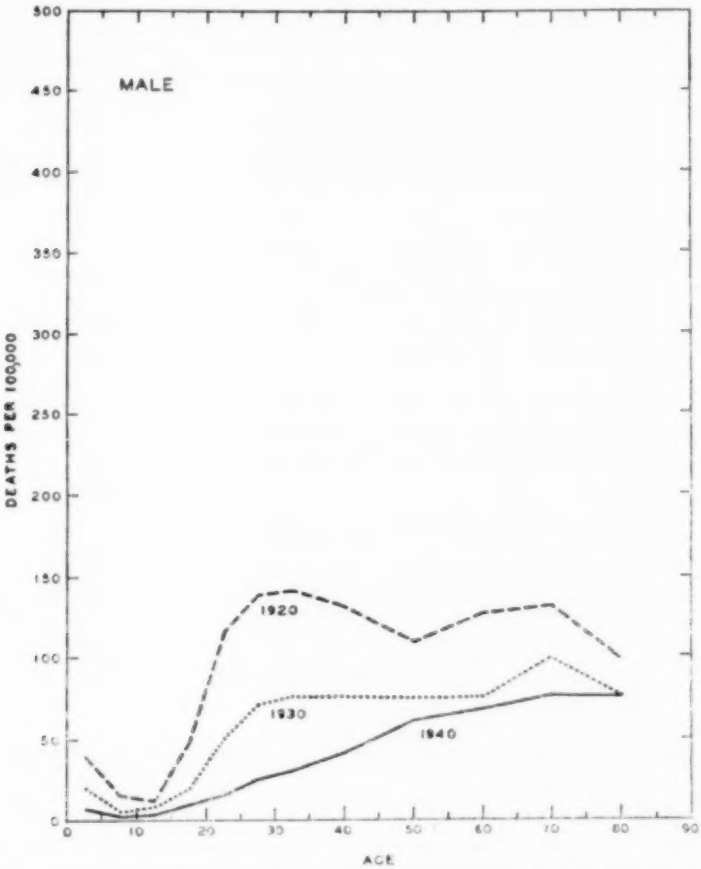

FIG. 9. Mortality rate per 100,000 in white males in Minnesota. Between 1920 and 1940 the death rate in white males under twelve years of age was higher in Minnesota than in North Carolina (Carroll E. Palmer, U. S. Public Health Service, 1943, vol. 1).

The reduction in the positive tuberculin rate in the school children in Minnesota for 1949 and 1950 to 3.5 per cent for the urban and 1.1 per cent for the rural schools is indeed a magnificent achievement (7). Unfortunately, no large-scale tuberculin testing has been carried out on the school children of North Carolina in recent years. It is my guess that the North Carolina rate is now appreciably higher than that of Minnesota.

After twelve years of age the death rate climbed more rapidly in North Caro-

lina than in Minnesota, reaching a peak of 180 at the age of twenty-five in 1920; a peak of 120 at the age of thirty in 1930; and a peak of 110 at the age of fifty in 1940. The corresponding peaks in Minnesota were 140 at the age of thirty in 1920; a peak of 80 at the age of seventy in 1930; and a peak of 100 at the age of eighty in 1940. The earlier peaks in North Carolina and the higher rates in old age, in the absence of a high infection rate in infancy and childhood, suggest that the white adult males of North Carolina are exposed to more tuberculous

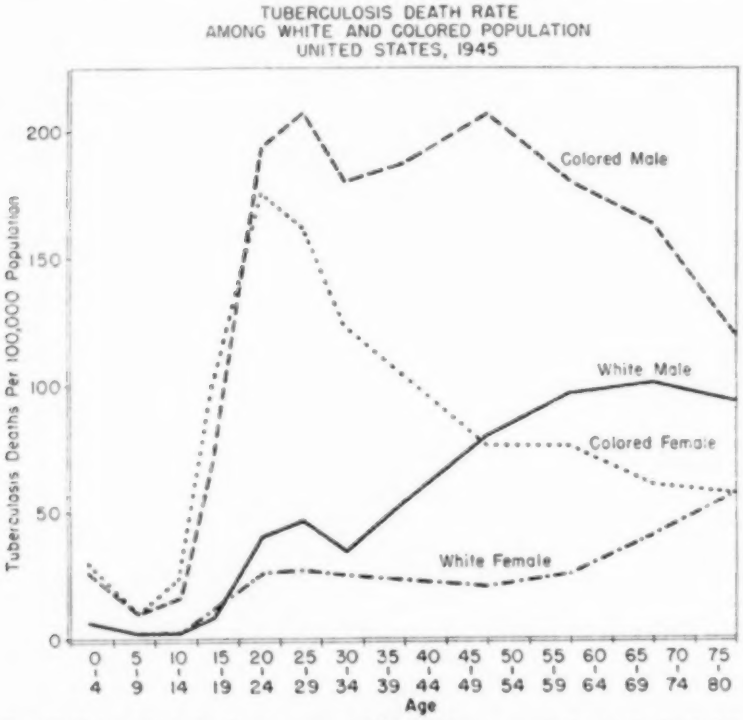

FIG. 10. Tuberculosis death rate among white and Negro population in the United States for the year 1945. Note the rising rate in old age, especially in white males. (Data supplied by Miss Dempsey from the Statistical Service of the National Tuberculosis Association.)

infections than those of Minnesota. This can be explained by the deficit in sanatorium beds in North Carolina. There have never been anywhere nearly enough beds in North Carolina to isolate all the known active "positive sputum" cases although we hope we will have enough when the new building program has been completed.

The decrease in infant mortality in Minnesota, the reduction in the percentage of school children with positive tuberculin tests, and the change in the shape of the death curves are convincing evidence that the major reduction in the death

rate in Minnesota was due to the vigorous control program and not to the general effect of a rising standard of living or other nonspecific factors.

THE VALUE OF THE TUBERCULIN TEST

Curves of the type shown in figure 10 are extremely valuable in showing which age and sex segment of the population has the highest death rate from tuberculosis, but the incidence of tuberculosis does not necessarily follow the same pattern. The incidence of newly detected cases is often merely a reflection of the vigor of the case-finding program, and no one can say with accuracy how long a patient, found by mass roentgenographic survey or other means, has had an active infection. If the incidence and death rate curves were supplemented by tuberculin curves which extended from infancy to old age, one could measure for the first time the extent of the residual tuberculous infection and predict where new cases would develop. As the death rate and, finally, the case rate decline, the tuberculin test becomes our most valuable case-finding method, both as a guide to the source of infection and as a label on those persons who will require annual roentgenograms to detect the first indication of active clinical disease.

When this stage of development is reached in the control of tuberculosis, the present type of mass roentgenographic survey can be eliminated and a new more selective type substituted which should reach every tuberculin-positive reactor in the state each year.

TUBERCULOSIS CAN BE ELIMINATED

A sufficiently vigorous program of the type outlined above should eliminate tuberculosis as a disease. Tuberculosis can be eliminated as surely as leprosy was eliminated from Western Europe between 1300 and 1600 A.D., but this cannot be accomplished in one generation. A child who acquires its tuberculous infection at the age of three, as shown by a positive tuberculin test, may not develop clinical tuberculosis until the age of eighty. As the final stages of the fight against tuberculosis are reached, the expense of hospitalization and treatment will be materially reduced but the expense of case finding, including tuberculin testing the entire population and roentgenographic examinations of all positive reactors annually, will be increased. Finally, when the number of tuberculin reactors becomes small, the cost of the annual roentgenogram will be insignificant. A little more investment in case finding now will save millions of dollars later in the prevention of new clinical cases which require expensive sanatorium treatment.

SUMMARY

The rapid, precipitous decrease in the death rate from tuberculosis since 1947 can be attributed primarily to improved medical and surgical treatment. The death rate can no longer be used as even a rough index of the frequency and importance of this disease. The decrease in death rate has not been accompanied by a corresponding decrease in the rate at which new cases are developing. There are reasons for believing that many active cases remain undetected in the population and form a source for new infections.

The remarkable progress in the control of tuberculosis in Minnesota is the direct result of the active control program and not the spontaneous effect of a rising standard of living or other nonspecific factors.

The control program for the future should be expanded to include a tuberculin test for every person in the state, with an annual roentgenogram of every positive reactor and an annual tuberculin test for every negative reactor. The money saved in treatment, by detecting the new cases in the incipient rather than in the moderately advanced stage of the disease, should be more than enough to pay for the increased cost of the expanded case-finding program when once the complete program has been activated.

SUMARIO

La Tuberculosis Hoy y Mañana

El rápido y precipitado descenso de la mortalidad tuberculosa desde 1947 puede atribuirse primordialmente a los perfeccionamientos del tratamiento médico y quirúrgico. Ya no cabe usar la mortalidad ni siquiera como índice aproximado de la frecuencia e importancia de la dolencia. La disminución de la mortalidad no ha ido acompañada de una disminución correspondiente de la velocidad con que aparecen nuevos casos. Hay motivos para creer que quedan muchos casos sin descubrir en la población, que sirven de focus para nuevas infecciones.

El notable adelanto observado en la lucha antituberculosa en el estado de Minnesota es consecuencia directa de un plan activo de lucha y no efecto espontáneo del mejoramiento de las condiciones de la vida o de otros factores inespecíficos.

El plan de lucha para el futuro debe ser expandido de modo que comprenda una prueba con tuberculina para todos los ciudadanos del estado, con una radiografía anual de todos los reactores y una prueba anual con tuberculina para todos los negativos. Lo economizado en tratamiento, por descubrir los casos nuevos en el período incipiente, más bien que en el moderadamente avanzado de la enfermedad, debe ser más que suficiente para cubrir el mayor costo del plan expandido de descubrimiento de casos, una vez puesto en efecto el plan en su totalidad.

RESUME

La Tuberculose Aujourd'hui et Demain

La chute brusque du taux de mortalité par tuberculose depuis 1947 peut être attribuée principalement à l'amélioration des traitements médicaux et chirurgicaux. Actuellement, il n'est plus possible de prendre le taux de mortalité comme un indice, même approximatif, de la fréquence et de l'importance de cette affection. L'abaissement des taux de mortalité ne coïncide pas avec une réduction correspondante de la fréquence d'apparition de cas nouveaux. Il y a des raisons permettant de croire qu'il y a dans la population un grand nombre de cas non-décelés qui sont à l'origine de nouvelles infections.

Les progrès remarquables réalisés dans le contrôle de la tuberculose dans l'Etat du Minnesota sont le résultat direct d'un programme de lutte anti-tuberculeuse activement poursuivi, et non l'effet spontané de l'amélioration du standard de vie ou de tout autre facteur non-spécifique.

Le programme anti-tuberculeux futur devrait être étendu jusqu'à comprendre: l'épreuve à la tuberculine chez tout sujet en résidence dans l'Etat, un radiogramme annuel chez tout individu ayant une réaction positive, et un tuberculine-test annuel pour tout individu anergique. L'argent épargné en thérapeutique, grâce à la détection de cas nouveaux au stade initial plutôt qu'au stade évolutif, serait plus que suffisant pour satisfaire aux dé-

penses accrues réclamées par l'extension du programme de détection de cas nouveaux, quand l'ensemble du programme sera réalisé.

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A METHOD FOR EVALUATING THE ATTITUDES OF TUBERCULOUS PATIENTS[1]

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INTRODUCTION

The attitudes and emotional reactions of the patient are important factors in the treatment of tuberculosis. Hartz (1), Prout (2), Wittkower (3), Coleman, Hurst, and Hornbein (4), Brooks (5), and others maintain that the best treatment results occur when attention is focused, not only on the diseased lungs, but also on the thoughts and feelings of the person in whom the lungs are confined. This view has probably been expressed most aptly by Osler's oft quoted remark: "It is just as important to know what is in a man's head as what is in his chest if you want to predict the outcome of his pulmonary tuberculosis."

The recalcitrant patient who refuses to conform to his bed-rest schedule is an obvious example of the way in which the course of treatment can be affected by "what's in a man's head." There is also the obstinate patient who is unwilling to accept the diagnosis of tuberculosis and refuses medication. In addition, one frequently observes the anxious patient who is highly fearful of his disease or worries constantly about the support of his family. The emotional state of these patients is hardly conducive to rest. Perhaps the most striking illustration of the effect of harmful attitudes upon medical treatment is seen when the patient suddenly leaves the hospital against medical advice. The seriousness of this problem has been stressed recently by Tollen (6), who has pointed out that more than half of the patient population in many hospitals receive "irregular discharges" before treatment is completed.

These interferences with medical treatment may be lessened considerably if the patient's attitudes are recognized and dealt with early in hospitalization. If, for example, it is discovered at the time of admission to the hospital that a patient is immature, lonely, and frightened of his disease, he may be helped by placement in a room next to a good-natured, older person. The patient who is distressed by family problems may need the immediate assistance of the social worker, whereas the person who finds bed-rest restrictions intolerable may benefit from the attention of the rehabilitation or special services sections. The severely maladjusted patient should be seen by the psychiatrist as soon as possible. Adjustment to hospital life can be made easier in many instances, and a great deal of restlessness, complaining, repeated requests for passes, and irregular discharges may be averted if one can spot harmful and irrational attitudes soon enough.

Ideally, an assessment of the mental and emotional aspects of the patient's illness should be undertaken as part of the admission medical history. In practice,

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however, this is rarely done. Many physicians lack the time, or they may feel uneasy about exploring the patient's emotional problems. An appraisal of the patient's attitudes is obtained, in some hospitals, through interviews by the medical social worker or psychiatrist. Such efforts, however, usually are quite time consuming. Too often, requests for the services of these specially trained interviewers take place *after* the patient's problems have become acute or unmanageable.

A need exists, therefore, for a reliable method of inquiry into the tuberculous patient's attitudes and emotional problems which readily lends itself to inclusion in routine hospital procedures. Such a method should require a minimum of time for administration, yet yield sufficient information to be of real assistance to the ward physician in understanding his patients.

An attitude form, designed to meet the above requirements, has been developed at the Veterans Administration Hospital in Madison, Wisconsin. This form has been administered to more than 200 tuberculous patients. A preliminary survey of the results indicates that the form promises to be a highly effective means of eliciting the patient's attitudes. Information obtained from individual records has been of considerable value to the hospital staff in dealing with the patient's adjustment to his disease and hospitalization.

OBSERVATIONS

The Sentence Completion Method

The attitude form employs the sentence completion method, a "projective technique" widely used by clinical psychologists in a variety of hospital settings. High validity for the sentence completion method has been claimed by Sacks and Levy (7) and Rotter and Willerman (8) when utilized as a measure of personal attitudes for patients in mental hygiene clinics and as a screening device in Army Air Force convalescent hospitals.

The sentence completion method consists of a number of incomplete sentences presented to a patient for completion; for example, "I don't like it when . . .," or "The future looks . . .". It has been found that by completing these sentences the patient often expresses his own wishes, desires, fears, likes, and dislikes. The standard administration of the form requires that the patient complete each item in writing. If the patient has difficulty in expressing himself in writing, one can administer the items orally and record the patient's verbal responses. The oral procedure may also be used when the examiner wishes to make a direct observation of the patient's behavior.

The sentence completion method has a number of advantages over the traditional "paper and pencil" type of questionnaire. The patient filling out a standard questionnaire can answer only "yes" or "no" to a series of questions. For example, in answer to the question, "Does lying in bed make you restless?", the patient can merely affirm or deny this feeling. The sentence completion method, however, permits a far greater freedom of responses. The patient is free to express his own personal feelings in any manner he wishes. If he is asked, for example,

to complete the sentence "Lying in bed makes me . . . ," he has a greater opportunity to express his *own* particular reaction to bed rest. One patient may regard bed rest as tiresome; another may state that lying in bed is the only way by which he can get better; whereas a third patient may feel that bed rest makes him nervous and irritable.

To convince oneself of the utility of the sentence completion technique, one may wish to duplicate the following simple experiment. An incomplete sentence was presented to ten male tuberculous patients selected at random: "When a person is first told he has tuberculosis . . ." Each patient was asked to read the sentence and to complete it as rapidly as possible. The following ten responses were obtained:

When a person is first told he has tuberculosis . . .

1. It is somewhat of a shock.
2. I didn't believe it.
3. He starts thinking of his family and how they will get along when he leaves.
4. He feels like the last man on "crack the whip."
5. SHOCK!!! Everything exploded in the air. This is the end.
6. It is disgusting, filthy.
7. Can it be possible?
8. It is not very good news.
9. He feels like an outcast.
10. It puts fear in one as to know how other people regard him as a friend.

Each of the ten statements reflects some degree of distress over the initial diagnosis of tuberculosis. Yet the individual differences in personal reactions, attitudes, and intensities of feeling are striking. Statements 1, 4, 5, and 8 all reflect the patient's displeasure upon being told of the disease. But even among these four responses there is considerable variation. On the one extreme there is the severe emotional upset evidenced in the statement, "SHOCK!!! Everything exploded in the air. This is the end." This patient appears to have reacted as if he were hit by a bomb and all is momentarily lost. In contrast with this open manifestation of uncontrolled emotionality are the responses "it is somewhat of a shock" and "it is not very good news." Such statements suggest a greater capacity for control over one's impulses. The emotional reactions are toned down and subdued almost to the point of understatement. Response 4, "feels like the last man on 'crack the whip'," gives us a clue to the specific fears and apprehensions felt by some patients. This sentence completion graphically portrays an attitude of precariousness, lack of support, and a fearful anticipation of being suddenly catapulted into isolation and unknown dangers. An overtone of morbid humor is also reflected in this response.

Responses 9 and 10 indicate how the disease may mean social rejection and ostracism for two of the patients. For them, the contagious aspect of the disease implies separation from friends and isolation from society. To feel "like an outcast" or to fear "how other people regard him as a friend" are common sentiments expressed by many tuberculous patients. Such attitudes are often accompanied by feelings of being regarded "as a leper" or being "locked up in a prison" while in the hospital. Response 6, "it is disgusting and filthy," mirrors

an attitude also held by some patients. Tuberculosis may be regarded by them as something unclean and shameful, perhaps comparable to venereal disease. This attitude is, at times, prompted by a deep-seated feeling of self-condemnation or a need for punishment for some "sinful" behavior in the past.

The patient who completes the sentence with "he starts thinking of his family and how they will get along when he leaves" is probably revealing a realistic concern for his family's welfare. On the other hand, the patient who "didn't believe it" apparently has refused to accept the initial diagnosis. Perhaps he was afraid to face the truth. Response 7, "can it be possible?" reflects a similar attitude of surprise and disbelief.

This small experiment involving the completion of a single sentence indicates some of the potentialities of the sentence completion method. It frequently is possible to form certain hypotheses about the patient's attitudes from the analysis of a single response. These initial impressions may then be verified or reinterpreted in the light of responses to additional sentences. The possibilities inherent in the completion of 70 to 80 sentences dealing with the patient's reactions to his disease and his hospitalization are quite impressive.

The Madison Sentence Completion Form

The Madison Sentence Completion Form was designed specifically to evaluate the attitudes and emotional reactions of tuberculous patients in nine significant areas of adjustment. The nine areas covered by the form include the patient's reactions toward (*1*) bed rest, (*2*) hospitalization, (*3*) tuberculosis, (*4*) ward life, (*5*) the medical staff, (*6*) irregular discharge, (*7*) family and friends, (*8*) medical treatment, and (*9*) attitudes of a general nature.

Construction of the Form

The Madison Sentence Completion Form was constructed in the following manner: Two hundred and fifty incomplete sentences were assembled by the present writer. Most of the sentences were based on attitudes expressed by hospital patients in interview sessions. To these were added sentences representing characteristic attitudes of patients, as indicated in the literature dealing with the emotional aspects of tuberculosis. A number of items were obtained through suggestions from ward physicians, nurses, and other hospital staff members. The 250 incomplete sentences were grouped into the nine attitudinal areas. A rating board of twelve judges, made up of two ward physicians, two nurses, two psychiatrists, three social workers, and three members of the administrative staff, rated each sentence on a three-point scale with reference to the potentiality of each sentence for eliciting the patient's attitudes in the various areas.

A preliminary sentence completion form, containing the 90 incomplete sentence items rated highest by the judges, was assembled and distributed to the entire hospital patient population for completion. Each of the 90 incomplete sentence items then were evaluated by a careful analysis and comparison of the test responses of two groups of patients. The first group consisted of 25 patients who

received irregular discharges, "against medical advice," within a six-month period after the initial distribution of the sentence completion forms. The second group consisted of 25 patients who were discharged during the same period, on a regular "maximum hospital benefit" basis.

Approximately one-third of the items were found to discriminate effectively between the two groups. These discriminating items were retained in the final version of the sentence completion form. Those items which did not discriminate between the two groups, but which yielded rich, idiosyncratic attitudes, were likewise retained. Those items which tended to produce stereotyped responses were eliminated. A number of the retained items were rephrased, and several new ones were added in order to obtain an optimal distribution of items within the nine attitudinal areas. The final version (reproduced below) consisted of 80 items.

The Adopted Form

The Madison Sentence Completion Form together with the introductory statement on the face sheet are reproduced below:

We are very much interested in knowing about how people feel when they have tuberculosis. You undoubtedly have many questions and feelings about your physical condition and the progress you are making toward recovery. You may have worries of one kind or another while you are in the hospital, either about your health, your family, your job or about many other things. You may feel one way or another about the hospital's rules and procedures or about the medical treatment.

By completing these sentences you can tell us how you actually feel about these matters. *There are no right or wrong answers, so feel free to write whatever you wish.* Your opinions will be held confidential. We believe that the information you give us can be of help in planning treatment in the future.

MADISON SENTENCE COMPLETION FORM

Name:——————— Ward:——— Class:——— Date:———

INSTRUCTIONS: Below are a number of incomplete sentences. By completing these sentences you can express how you feel about many things that concern you while you are in the hospital. Finish each sentence as well as you can. Feel free to write whatever you wish.

1. Lying in bed makes me
2. While I am here
3. When a person is first told he has tuberculosis
4. The thing that helps most to pass the time
5. I like a doctor who
6. I feel like going home when
7. While I am in the hospital, my friends
8. When a patient is told he needs surgery
9. When the odds are against you
10. When other people take care of me
11. When a patient has to stay in bed for a long time
12. The rules and regulations of this hospital
13. When people know you have TB
14. A good roommate is one who
15. I think that the doctor
16. If I were to leave the hospital now
17. Visitors
18. Waiting for surgery
19. Since coming here, my religion
20. My biggest fear
21. Being here in bed all day
22. What I like best here is
23. Tuberculosis can be
24. When I listen to the radio
25. I would feel better if the doctor
26. I will leave the hospital, if
27. While I am in the hospital, my family
28. After a person has surgery

29. The sort of person I like the most is
30. A person can use a drink when
31. I believe I should be in class ——— because
32. What I don't like here is
33. When someone enters my room wearing a mask
34. The recreation and entertainment
35. When the doctor talks about my case
36. A patient feels like walking out of here
37. The social worker
38. The strep shots
39. The sort of person I don't like is
40. I worry most about
41. When the nurse says "Get back in bed!"
42. This hospital is like
43. Maybe I wouldn't have gotten sick if
44. When a patient moves to another ward
45. My doctor
46. I expect to stay here until
47. My family responsibilities
48. The new drugs used for tuberculosis
49. A patient's sex life
50. Filling out a form like this
51. The future looks
52. The patient's main gripe is
53. Having TB wouldn't be so bad if
54. The hospital meals
55. I like a nurse who
56. The best judge of when I should leave here is
57. The person I miss the most while I am here
58. What really helps my cure the most is
59. A real friend
60. This sentence completion form
61. After I get out of the hospital
62. What I'll miss the most when I leave the hospital
63. The worst thing about having tuberculosis is
64. The O. T. worker
65. The nurses
66. The length of time I plan to stay in the hospital is
67. When a patient is away from his job
68. When a patient's case is brought up before the therapy board
69. When people push me around
70. The psychologist
71. My plans for the future
72. When a patient asks for a pass and doesn't get it
73. What I like best when I'm sick
74. I prefer / do not prefer (cross out one) to be alone in my room
75. I have most confidence in
76. I expect my cure to take about ——— months
77. A wife should
78. I would feel better if
79. I feel happiest when
80. In order to make this a better hospital to be in, I suggest that

The 80 items on the Madison Sentence Completion Form are arranged in a particular sequence in order to facilitate the examination of the patient's responses with respect to each of the attitudinal areas. For example, all the numbers ending in the digit "5" (items 5, 15, 25, et cetera) identify sentences dealing with attitudes toward the medical staff. In a similar manner each of the other attitudinal areas are identified by certain item numbers. The division of the items into the nine attitudinal areas is as follows:

Attitudes toward bed rest: Items 1, 11, 21, 31, and 41 deal with bed rest. These items are intended to elicit responses indicative of the patient's reactions to continuous confinement in bed.

Attitudes toward hospitalization: Items 2, 12, 22, 32, 42, 52, 62, 72, and 80 are aimed at revealing the patient's feelings about hospital living. Included in this area are three items (51, 61, and 71) which bear on the patient's plans for the future, after leaving the hospital.

Attitudes toward tuberculosis: Items 3, 13, 23, 33, 43, 53, 63, and 73 pertain to the patient's reactions to his disease. They include incomplete sentences that permit the patient to express his attitudes toward the initial diagnosis of tuber-

culosis, as well as the particular significance the disease has for him while under treatment.

Attitudes toward ward life: Items 4, 14, 24, 34, 44, 54, 64, and 74 cover the patient's likes and dislikes concerning specific ward activities.

Attitudes toward the medical staff: Items 5, 15, 25, 35, 45, 65, and 75 give evidence of the patient's attitudes toward the doctors and the nursing staff.

Attitudes toward irregular discharge: Items 6, 16, 26, 36, 46, 56, 66, and 76 give clues as to the possibility that a patient may leave the hospital prematurely and against medical judgment.

Attitudes toward family and friends: Items 7, 17, 27, 37, 47, 57, 67, and 77 deal with the patient's family and social problems.

Attitudes toward medical treatment: Items 8, 18, 28, 38, 48, 58, 68, and 78 are related to the patient's reactions to surgery, drug therapy, and other phases of medical treatment.

Attitudes of a general nature: Items 9, 19, 29, 39, 49, 59, 69, 79, 10, 20, 30, and 40 are of a general nature. These items are intended to reveal the patient's attitudes toward dependency, his specific fears and worries, as well as his particular manner of reacting to frustrating situations. Also included in this area are items 50, 60, and 70, which give evidence of the patient's impressions of the tester and his over-all attitudes toward filling out the form.

Analysis and Interpretation

After the patient completes each sentence on the form, his responses are reassembled and transcribed to an analysis sheet. This sheet brings together under each of the nine attitudinal areas the specific items and the patient's responses to them. For example, the responses of a patient to items 6, 16, 26, 36, 46, 56, 66, and 76, dealing with the attitudes toward leaving the hospital prematurely, are brought together on the analysis sheet in the following manner:

Attitudes toward irregular discharge:

Item

6. I feel like going home when . . . *the Doctor tells me I am cured completely.*
16. If I were to leave the hospital now . . . *I'd just be hurting myself.*
26. I will leave the hospital if . . . *and when the Doctor says it's O.K.*
36. A patient feels like walking out of here when . . . *one who thinks like that should not come here in the first place. Am I not right?*
46. I expect to stay here until . . . *the cure comes.*
56. The best judge of when I should leave here is . . . *entirely up to the Doctor.*
66. The length of time I plan to stay in the hospital is . . . *a long time. The Doctor knows best.*
76. I expect my cure to take about *16* months . . . *I hope so anyway.*

The italicized words are the patient's responses. These responses are considered together and an interpretation is made, based on the examiner's impression of the patient's attitude toward irregular discharge. Similar interpretations are made for attitudes in each of the other eight areas. The over-all interpretive summary consists of an integration of the interpretations for the nine areas. Following is the interpretation given for the above example:

The patient appears to be a submissive, agreeable individual whose motto is "the Doctor knows best." He is anxious to please and obey the doctor. He emphatically states that he will remain in the hospital until "the Doctor tells me I am cured completely" and he will leave "if and when the Doctor says it's O.K." He realizes that an irregular discharge is detrimental to the patient's welfare. In view of the patient's strong faith in the doctor's judgment and his eagerness to conform to hospital procedures it seems unlikely that he will leave the hospital against the advice of the medical staff.

The interpretation of the sentence completion responses shown above may be compared with the one given for the following set of responses offered by a second patient:

Attitudes toward irregular discharge:

Item

6. I feel like going home when . . . *they tell me get back in bed everytime they look at you.*
16. If I were to leave the hospital now . . . *I'd be my own boss.*
26. I will leave the hospital if . . . *whenever I feel I am in good enough shape.*
36. A patient feels like walking out of here when . . . *he gets good and disgusted.*
46. I expect to stay here until . . . *as long as I can possibly take it, and then take off.*
56. The best judge of when I should leave here is . . . *myself and the thinking I do.*
66. The length of time I plan to stay in the hospital is . . . *a few more months at the most.*
76. I expect my cure to take about — months . . . (leaves blank)

The patient appears to be an outspoken, independent person who finds hospital restrictions highly intolerable. He places a high premium on being "my own boss," doing his own thinking and making his own decisions, rather than rely on the judgment of the medical staff. He intimates that he will leave the hospital whenever *he* feels he is "in good enough shape" or when he "gets good and disgusted." The patient's belligerent attitude and his apparent unwillingness to accept a dependent-patient role makes him a likely candidate for irregular discharge.

The interpretations given above correspond very closely with the independent judgments of the two patients which were made by physicians and ward nurses. The first patient, who felt that "Doctor knows best," consistently behaved in a "model" fashion while at the hospital. He subsequently received his regular discharge. The second patient, who stated that he wished to "be my own boss" and who suggested that one should stay in the hospital as long as "he can possibly take it—and then take off," actually did leave the hospital against medical advice one month after the administration of the sentence completion form.

COMMENT

There are no rigid rules of interpretation for the Madison Sentence Completion Form. A flexible clinical approach is recommended.[2] In general, the sentence completion responses can be accepted at their face value as being indicative of how the patient actually feels. Occasionally, however, a patient may be on

[2] An objective scoring system, in which a scale value is assigned to each sentence completion response, is being developed at present. An over-all hospital adjustment score for each patient can be obtained by this method. The objective scoring system may have particular value in experimental studies and in screening for potential irregular discharges among patients.

his guard and offer only evasive or stereotyped sentence completions. These attempts to hide one's true feelings do not necessarily invalidate the record. Evasive answers often indicate a suspicious, distrustful person who is afraid of revealing his inadequacies. This type of person usually is unwilling to confide in his doctor and frequently is unable to form warm personal friendships with other patients on the ward.

Brief inspection of the sentence completion record ordinarily discloses much useful information regarding the patient's day-to-day attitudes. The more skilled and experienced the interpreter, however, the greater the yield of pertinent and valuable data. The interested physician, social worker, or clinical psychologist should be able to obtain a fairly comprehensive picture of the patient's personality based on the responses to the sentence completion form.

SUMMARY

A psychological form (the Madison Sentence Completion Form) has been designed specifically to evaluate the attitudes and emotional reactions of tuberculous patients in nine significant areas of adjustment. By completing a series of 80 incomplete sentences the patient expresses his attitudes and feelings in regard to (*1*) bed rest, (*2*) hospitalization, (*3*) tuberculosis, (*4*) ward life, (*5*) the medical staff, (*6*) irregular discharge, (*7*) family and friends, (*8*) medical treatment, and (*9*) attitudes of a general nature.

The form is administered easily and requires a minimum of time and supervision. Information obtained from the form can be of real assistance to the hospital staff in understanding the emotional and social problems of the patient. Specifically, the Madison Sentence Completion Form can be useful in detecting harmful attitudes which seriously interfere with medical treatment or which lead to irregular discharge.

SUMARIO

Método para Apreciar la Actitud de los Tuberculosos

Un formulario psicológico (Formulario Madison para Completar Oraciones) ha sido confeccionado específicamente para justipreciar la actitud y las reacciones afectivas de los tuberculosos en nueve importantes segmentos de adaptación. Al completar una serie de 80 oraciones incompletas, el enfermo expresa su actitud y sentimientos con respecto a: (*1*) reposo en cama, (*2*) hospitalización, (*3*) tuberculosis, (*4*) vida en las salas del hospital, (*5*) personal médico, (*6*) alta irregular, (*7*) familia y amigos, (*8*) tratamiento médico, y (*9*) actitud en general.

El formulario se utiliza fácilmente y requiere un mínimo de tiempo y de vigilancia. La información obtenida con el mismo puede ser de ayuda real al personal hospitalario para comprender los problemas afectivos y sociales del paciente. Específicamente, el Formulario Madison para Completar Oraciones puede resultar útil para descubrir actitudes nocivas que afectan seriamente el tratamiento médico o que conducen a altas intempestivas.

RESUME

Une Technique d'Évaluation des Attitudes du Malade Tuberculeux

Un questionnaire représentant un test psychologique (Madison Sentence Completion Form) a été préparé dans le but spécifique d'évaluer les attitudes et les réactions émotio-

nelles du malade tuberculeux dans 9 domaines de l'adaptation. En complétant une série de 80 phrases incomplètes, le malade exprime son attitude et ses sentiments concernant: (*1*) le repos au lit, (*2*) l'hospitalisation, (*3*) la tuberculose, (*4*) al vie dans la salle d'hôpital, (*5*) le personnel médical, (*6*) le départ de l'hôpital, non-autorisé médicalement, (7) la famille et les amis; (*8*) le traitement médical, (*9*) le comportement en général.

Cette fiche qui est facilement établie ne reclame qu'un minimum de temps et de surveillance. Les renseignements fournis par cette fiche peuvent constituer une aide réelle pour les différentes catégories du personnel hospitalier en leur permettant de comprendre les problemes émotionnels et sociaux du malade. Plus spécifiquement la "Madison Sentence Completion Form" peut servir à déceler les attitudes nocives qui gênent considérablement le traitement médical ou conduisent au départ prematuré du sanatorium.

Acknowledgments

The writer wishes to express his appreciation to Dr. Thomas H. Lorenz, Acting Chief, Psychosomatic Section, and to Dr. William C. Lewis, Psychiatric Consultant, of the Veterans Administration Hospital, Madison, Wisconsin, for their helpful suggestions in the construction of the Madison Sentence Completion Form and in the writing of this paper.

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OBSERVATIONS ON THE EFFECT OF INDUCED HYPERGLYCEMIA ON THE GLUCOSE CONTENT OF THE CEREBROSPINAL FLUID IN PATIENTS WITH TUBERCULOUS MENINGITIS[1,2]

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INTRODUCTION

Because of the high incidence of block and hydrocephalus in patients with tuberculous meningitis who do not respond to therapy, the present writers became interested in investigating the blood-cerebrospinal fluid barrier in an attempt to find a test which would facilitate early diagnosis of either of these two major complications and, perhaps at the same time, clarify some of the abnormalities which occur in the cerebrospinal fluid constituents.

The streptomycin diffusion index[5] has been of great value on this service in the diagnosis of spinal block in tuberculous meningitis, but there is a delay of approximately thirty-six hours in obtaining the results. Pneumoencephalography is a valuable aid in the diagnosis of hydrocephalus and block; however, it involves certain risks and is of little value in the diagnosis of early block.

Wilcox, Lyttle, and Hearn (2) stated that "the change in the permeability of the choroid plexus would seem to be the logical explanation for many of the changes in the composition of spinal fluid in disease." This opinion, which was emphasized also by Cohen and Libman (3) and other workers, stimulated the present writers to investigate the permeability of the blood-cerebrospinal fluid barrier as an indicator of pathologic changes in the central nervous system.

It is well known that the cerebrospinal fluid glucose content changes in certain diseases, being low in the purulent meningitides, hypoglycemia, and tuberculous meningitis; and high in diabetes mellitus. The amount of rise or fall in the concentration of glucose in the cerebrospinal fluid reflects the concentration of sugar in the blood, the difference between the two apparently being due to selectivity on the part of the barrier between the blood and the cerebrospinal fluid.

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[2] This study was aided by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; from Parke, Davis & Co.; and from the Division of Research Grants and Fellowships of the National Institutes of Health, Public Health Service.

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[5] A streptomycin diffusion index (I) is the ratio of cisternal over lumbar streptomycin levels taken eight hours after the injection of 30 mg. of streptomycin in the lumbar region. Normally it should be 1.0. In the presence of partial block, the index is less than 1.0. In the presence of complete block, the index is 0.

The changes in the cerebrospinal fluid glucose in tuberculous meningitis have not been adequately explained. Three factors may be responsible for the low glucose:

(*A*) consumption of glucose by leukocytes and bacteria

(*B*) glycolysis due to bacterial enzymes

(*C*) decreased permeability of the blood-cerebrospinal fluid barrier

Goldring and Harford (4) and Weichsel and Herzger (5), as a result of experiments they conducted, believe that there is insufficient evidence even in bacterial meningitis to support the theory that the lowering of the cerebrospinal fluid glucose is caused entirely by consumption of the glucose by leukocytes and bacteria. It seems possible that a common factor may be at least partly responsible for the lowering of the cerebrospinal fluid sugar in tuberculous and other pyogenic meningitides. Grayzel and Orent (6) showed that, when glucose was introduced into the peritoneal cavity of dogs, the glucose passed from the blood into the cerebrospinal fluid, producing a hyperglycorrhachia which reflected the rise in the blood sugar but was delayed. The maximum rise obtained in the cerebrospinal fluid glucose curves occurred in two to three hours. After the peak was reached, there was a slow fall to normal in six to seven hours. Fremont-Smith and Dailey (7) and many others carried out tests with intravenous glucose in patients, and their results paralleled the findings of Orent and Grayzel in dogs. Mehrtens and Newman (8) performed similar studies in patients, using alcohol by the intravenous route, and determined the concentration of the alcohol in the cerebrospinal fluid in both the lumbar and cisternal regions. They obtained higher concentrations in the cistern than in the lumbar region. More recently, Sweet and his co-workers (11) have studied the barrier by using radioactive sodium and heavy water. They noted that the concentration of radioactive sodium was greatest in the ventricle and least in the lumbar region. Heavy water diffused into all portions of the cerebrospinal fluid but reached the highest concentration in the cistern.

In the present study, an attempt was made to find a substance which would be harmless and which would pass readily from the blood into the cerebrospinal fluid. Substances such as glucose, urea, and creatinine given by the oral route did not appear in the cerebrospinal fluid in significant amounts. Hypertonic solutions of sodium chloride injected intravenously failed to increase the cerebrospinal fluid chlorides. Glucose administered intravenously passed readily into the cerebrospinal fluid, was harmless, and could be measured accurately. For these reasons it was chosen for the present writers' purposes.

MATERIALS AND METHODS

The patients received nothing by mouth for six hours. Adequate sedation was obtained by giving sodium phenobarbital, 3 to 5 mg. per kg., one and one-half hours before the test. The hair was carefully shaved from the nape of the neck, and the cisternal and lumbar areas were cleaned with soap and water followed by alcohol. Glucose was then given intravenously for a three- to five-minute period in a 20 per cent solution, in a dose of 1 gm. per kg. of body weight. The skin was cleaned a second time with alcohol and, with the patient in the lateral recumbent position, needles were introduced into the subarachnoid space

of the cisternal and lumbar regions, using a careful aseptic technique. In addition, ventricular punctures were performed whenever possible. Small amounts of fluid (approximately 0.5 cc.) were removed at fifteen-minute intervals during a period of two hours. Two persons were at hand at all times to watch the patient and to carry out the test satisfactorily. Water by mouth was offered during the test (120 to 240 cc.). The cerebrospinal fluid was precipitated by dilute tungstic acid as soon as possible after the samples were withdrawn. A modified Folin Wu micro method was used to estimate the glucose in the cerebrospinal fluid.

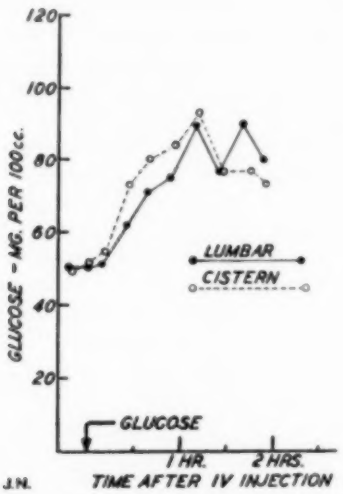

FIG. 1. (J. N.) Glucose test of a control patient. Both the cisternal and lumbar curves rise to a maximum approximately seventy minutes after the glucose injection. The cisternal curve is above the lumbar curve except toward the end of the two-hour period. The maximum rise of both curves is greater than 30 mg. per 100 ml. above the initial values. This graph is characteristic of the glucose test in the normal person.

For the sake of brevity, the test described will hereafter be designated as the *glucose test*, and the resulting elevations in the cerebrospinal fluid glucose will be referred to as *glucose curves*.

RESULTS

The study was carried out in the Children's Medical Service of Bellevue Hospital. Up to the present time sixty-five tests have been done. These tests were performed in children with no known disease of the central nervous system, in children with congenital hydrocephalus, and in children with tuberculous meningitis. The results of tests in children with congenital hydrocephalus will be reported elsewhere (10). The present report will be limited to observations in patients with tuberculous meningitis and in patients with primary tuberculosis who served as controls.

The tests have been divided into three groups, described below:

Group I: Controls: The control group included six tests on 5 patients with pulmonary tuberculosis who exhibited transient symptoms, such as unexplained fever and hyperreflexia, in whom the test was performed in an attempt to diagnose tuberculous meningitis. Further observation and investigation in each of these cases excluded the diagnosis. The cerebrospinal fluid was normal on the day of the test in each of these cases.

The curves of patients in *Group I* showed the following characteristic findings (as shown in figure 1).

> Both the cisternal and the lumbar glucose rose to a maximum level in the period between forty-five minutes and two hours after the injection of glucose. The cisternal glucose values were consistently higher than the lumbar values except towards the end of the two-hour period, when the lumbar glucose curve usually rose above the cisternal curve. The maximum rise above the lowest level of the lumbar and cisternal glucose during the time of the test was always greater than 30 mg. per 100 ml. and less than 60 mg. per 100 ml.

Group II: This group consisted of 6 patients recovering from tuberculous meningitis whose cerebrospinal fluid had been normal for periods ranging from two to seven months. Pneumoencephalograms were normal in all of these patients. The group showed glucose curves with the same characteristics as those described in *Group I*.

Group III: In the third group were patients with active tuberculous meningitis and patients with hydrocephalus resulting from tuberculous meningitis. The curves in these patients are described according to their variations from the appearance and values of the curves in *Groups I* and *II*, which are considered normal. A curve is considered abnormal if the values are higher or lower than the limits of *Group I* and if the normal relationship between cisternal and lumbar curves is reversed so that the lumbar curve is above the cisternal curve.

Case Reports

Patient G. W. (figure 2), a 10-year-old girl, was admitted on July 23, 1952, with early tuberculous meningitis and miliary tuberculosis. The cerebrospinal fluid contained 200 leukocytes per cu. mm., all lymphocytes; 24 mg. of glucose per 100 ml.; 50 mg. of protein per 100 ml. At this time the glucose test was performed before treatment of tuberculous meningitis was started. A pneumoencephalogram made one week after the glucose test was normal.

As may be seen in figure 2, the relationship between the cisternal and lumbar curves was normal, although the gap between the two was wider than usual. The rise of the cisternal curve above initial values was at the upper limits of normal, whereas the rise of the lumbar curve was at the lower limits of normal.

Patient F. M. (figure 3), a boy two and one-half years old, was admitted on July 30, 1952, with early tuberculous meningitis. The cerebrospinal fluid contained 58 leukocytes per cu. mm., of which 100 per cent were lymphocytes; 33 mg. of glucose per 100 ml.; and

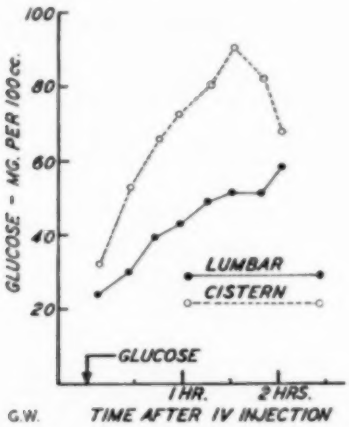

FIG. 2 (G. W.). Glucose test in a 10-year-old girl with early tuberculous meningitis before treatment. Notice the wide gap between cisternal and lumbar glucose curves, although the relationship is normal.

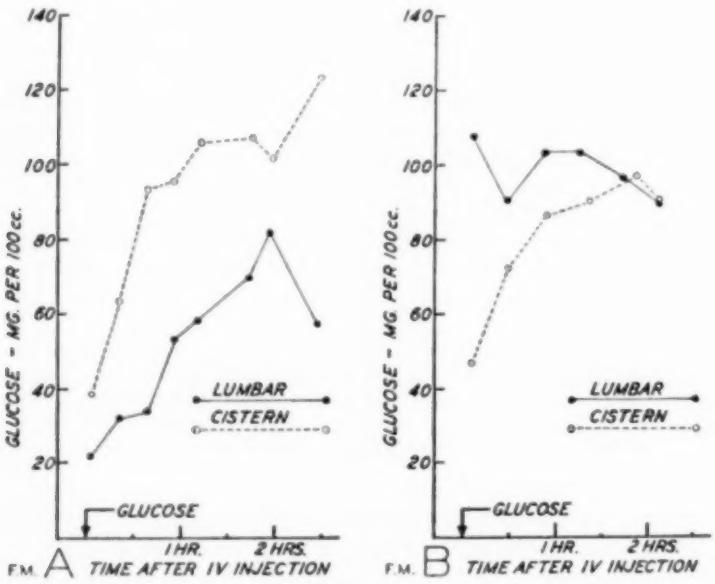

FIG. 3 (F. M.). Glucose tests in a 2-year-old boy with early tuberculous meningitis: (*A*) Before treatment was started; the relationship between the cisternal and lumbar curves is normal. The cisternal curve is abnormally high. There is a wide gap between the cisternal and lumbar curves. (*B*) Six days after the beginning of intrathecal therapy; the normal relationship between the cisternal and lumbar curves is reversed. The lumbar curve is unusually high at the beginning of the test.

113 mg. of protein per 100 ml. Cisternal and lumbar streptomycin concentrations[6] were 2.5 γ per ml. The first test (*A*) was done at this time. Treatment of tuberculous meningitis was started immediately afterwards, including intrathecal streptomycin, 100 mg. daily. A week later, when the second test (*B*) was done, the fluid contained 110 leukocytes per cu. mm., of which 100 per cent were lymphocytes; 47 mg. of glucose per 100 ml.; 102 mg. of protein per 100 ml.; and a streptomycin concentration of 2.5 γ per ml.

In the first test (*A*) (figure 3*A*), the relationship between the cisternal and lumbar curves was normal, but the gap between the two was wider than usual. The rise of the cisternal curve above initial levels was abnormally high. The rise in the lumbar curve was at the upper limits of normal.

The normal relationship between cisternal and lumbar curves was reversed in the second test (*B*) (figure 3*B*). The rise in the cisternal curve was within normal limits. The lumbar curve appeared elevated at the beginning of the test. Probably this represented a rapid elevation in the lumbar glucose taking place in the interval between the injection of glucose and the lumbar puncture since the fasting cerebrospinal fluid glucose on that day was 47 mg. per 100 ml.

Patient S. O. (figure 4), a 2-year-old girl, was admitted on July 10, 1952, with a moderately advanced tuberculous meningitis and miliary tuberculosis. The cisternal fluid contained 100 leukocytes per cu. mm., of which 90 per cent were lymphocytes; 39 mg. of glucose per 100 ml.; and 61 mg. of protein per 100 ml.; a streptomycin concentration of 1.25 γ per ml.; and *M. tuberculosis* on culture. The fluid obtained by lumbar puncture was bloody and contained 41 mg. of glucose per 100 ml., 80 mg. of total protein per 100 ml., and a streptomycin concentration of 1.25 γ per ml. The first *glucose test* (*A*) was performed on admission, and daily treatment with streptomycin (by the intramuscular and intrathecal routes), isoniazid, and Promizole was started immediately afterwards. Five days later the second test (*B*) was performed. At this time the cerebrospinal fluid contained 550 leukocytes per cu. mm., of which 75 per cent were lymphocytes; 39 mg. of glucose per 100 ml.; and 81 mg. of protein per 100 ml. A pneumoencephalogram on July 16 showed a moderate communicating hydrocephalus. The cerebrospinal fluid at the time of the third test (*C*) on July 22 contained 100 leukocytes per cu. mm., of which 90 per cent were lymphocytes; 55 mg. of glucose per 100 ml.; and 122 mg. of protein per 100 ml. The streptomycin diffusion index was 1.0. Six weeks after the third test, the patient continues to improve slowly.

In figure 4 it may be seen that the normal relationship between cisternal and lumbar curves was altered at the beginning of the test; however, this abnormality may have been due to bloody cerebrospinal fluid obtained during the early part of the test. The rise of the cisternal curve was abnormally low and the rise of the lumbar curve was above normal.

The normal relationship between the cisternal and lumbar glucose curves was reversed, as may be seen in figure 4*B*. Both cisternal and lumbar curves failed to show a significant rise.

In figure 4*C*, it may be seen that the normal relationship between the cisternal and lumbar glucose curves was markedly altered. The rise of the lumbar curve was within normal limits. The cisternal curve failed to show a significant rise.

[6] All streptomycin concentrations were determined in cerebrospinal fluid obtained two to three hours after the injection of 1 gm. of streptomycin by the intramuscular route. In patients who received intrathecal streptomycin, the levels were obtained twenty-four hours after the last intrathecal dose.

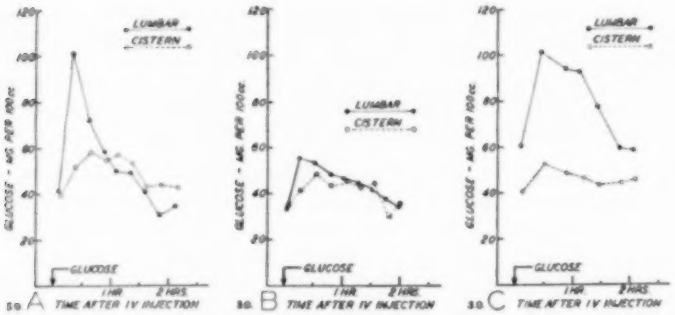

FIG. 4 (S. O.). Glucose tests in a 2-year-old girl with moderately advanced tuberculous meningitis: (*A*) Before treatment was started; the normal relationship between the lumbar and cisternal curves is altered in the early part of the test, possibly due to bloody cerebrospinal fluid obtained during the early part of the test. The rise of the cisternal curve is abnormally low. (*B*) Five days after beginning intrathecal therapy; the normal relationship between the cisternal and lumbar curves is reversed. The rise of both curves is abnormally low. (*C*) A week later; the normal relationship between the cisternal and lumbar curves is markedly altered. The lumbar rise is normal and the cisternal curve shows only a slight rise.

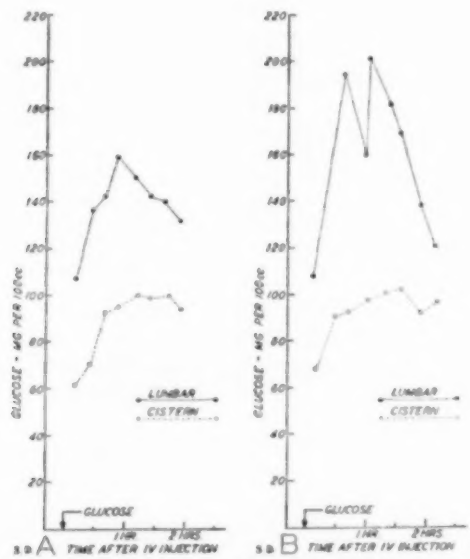

FIG. 5 (S. D.). Glucose tests of a 13-year-old girl with moderately advanced tuberculous meningitis: (*A*) Before therapy; shows normal rise in both curves, but the normal relationship between the lumbar and cisternal curves is already reversed. (*B*) After seven weeks of therapy; the curves show little change except that the elevation of the lumbar curve has become markedly accentuated.

Patient S. D. (figure 5), a 13-year-old girl, was admitted on June 11, 1952, with moderately advanced tuberculous meningitis. The cerebrospinal fluid contained 450 leukocytes per cu. mm., 100 per cent of which were lymphoycytes; 35 mg. of glucose per 100 ml.; 326 mg. of protein per 100 ml. The first glucose test (*A*) was performed on admission before treatment was started with Promizole and isoniazid, intramuscular streptomycin, and daily intrathecal streptomycin (100 mg.). The patient improved very gradually; the cerebrospinal fluid was xanthochromic, with proteins averaging 350 mg. per 100 ml. A streptomycin diffusion index on June 14, 1952, was 1.0. A pneumoencephalogram on the fifth day of treatment was normal. However, the cerebrospinal fluid pressure remained very low and the protein values high. On July 28, 1952, the cerebrospinal fluid contained 35 leukocytes per cu. mm., 90 per cent of which were lymphocytes; 40 mg. of glucose per 100 ml.; 450 mg. of protein per 100 ml. The following day the second glucose test (*B*) was performed. The patient continues to improve slowly.

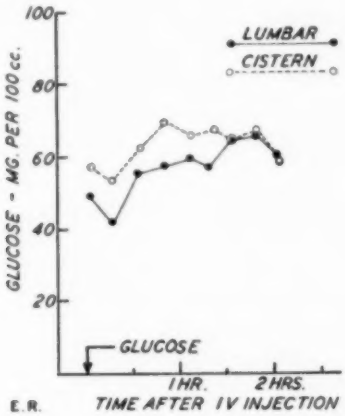

FIG. 6 (E. R.). Glucose test in a 14-month-old girl with moderately advanced tuberculous meningitis, showing normal relationship between cisternal and lumbar curves. Both fail to show a normal rise.

The normal relationship between the cisternal and lumbar glucose curves was markedly altered (figure 5*A*). The rise of both curves above initial values was within normal limits.

The abnormality in the relationship between the cisternal and lumbar curves became accentuated (figure 5*B*). The rise of the lumbar curve was considerably greater than the upper limits of normal. The rise of the cisternal curve was at the lower limits of normal.

Patient E. R. (figure 6), a 14-month-old girl, was admitted on August 30, 1952, with moderately advanced tuberculous meningitis. The cisternal fluid showed 87 leukocytes per cu. mm., 100 per cent of which were lymphocytes; 53 mg. of glucose per 100 ml.; 89 mg. of protein per 100 ml. The lumbar fluid had 70 leukocytes, 100 per cent of which were lymphocytes; 42 mg. of glucose per 100 ml.; and 93 mg. of protein per 100 ml. The glucose test was performed before treatment was started. A pneumoencephalogram performed five days after the glucose test showed the lateral ventricles to be within the upper limits of normal.

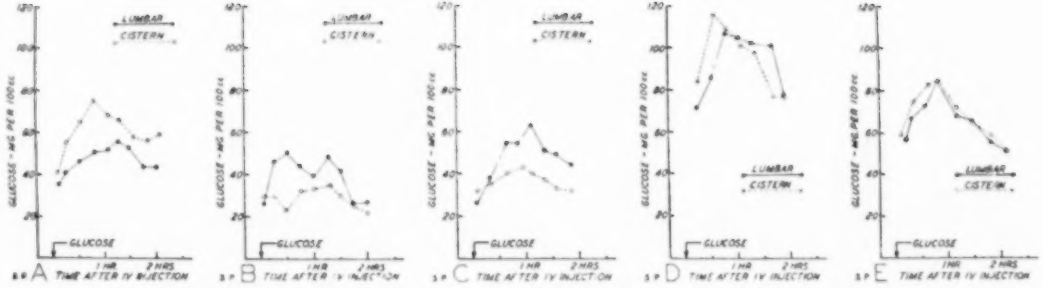

FIG. 7 (S. P.). Glucose tests in a 2-year-old boy with moderately advanced tuberculous meningitis: (*A*) Before treatment; shows normal relationship between cisternal and lumbar curves and an abnormally low lumbar rise. (*B*) After six days of treatment; reversal of the normal relationship between cisternal and lumbar curves has occurred. Both curves are abnormally low. (*C*) Two days later; there is little change except that the lumbar curve rises to normal values. (*D* and *E*) After seven and twelve weeks on therapy; curves are within normal limits.

The relationship between cisternal and lumbar curves was normal, but both curves failed to show a significant rise.

Patient S. P. (figure 7), a 2-year-old boy, was admitted on April 22, 1952, with a moderately advanced tuberculous meningitis. On admission the cerebrospinal fluid contained 340 leukocytes per cu. mm., 90 per cent of which were lymphocytes; 100 mg. of protein per 100 ml.; 25 mg. of glucose per 100 ml.; the culture was positive for *M. tuberculosis*. The first glucose test (*A*) was carried out at this time and treatment was started immediately afterwards with intramuscular and intrathecal streptomycin, 100 mg. daily. Six days later, when the second glucose test (*B*) was done, the cerebrospinal fluid findings were as follows: 230 leukocytes per cu. mm., 90 per cent of which were lymphocytes; 21 mg. of glucose per 100 ml.; 136 mg. of protein per 100 ml. A third glucose test (*C*) was performed on April 30, 1952; the cerebrospinal fluid findings were 40 leukocytes per cu. mm., 100 per cent of which were lymphocytes; 35 mg. of glucose per 100 ml.; 122 mg. of protein per 100 ml. A pneumoencephalogram on May 2, 1952, was normal. The patient improved, and the fourth and fifth tests (*D* and *E*) were performed on June 8 and July 10, 1952, while the patient was still on daily intrathecal streptomycin and the cerebrospinal fluid glucose values were between 38 and 40 mg. per 100 ml.

The relationship between the cisternal and lumbar curves was normal (figure 7*A*). The rise in the cisternal curve was within normal limits, i.e., more than 30 mg. per 100 ml. However, the rise in the lumbar curve was below normal.

A marked alteration in the relationship between the cisternal and lumbar curves had occurred by the time of the second test (figure 7*B*). The lumbar curve was above the cisternal; however, the rise of both curves above initial values was below normal.

There was little change from test *B* except that the rise in the lumbar curve was within normal limits (figure 7*C*). The cisternal rise above initial values was still abnormally low.

The relationship between cisternal and lumbar curves was within normal limits. The rise of both curves was also normal (figure 7*D*).

The initial glucose values were lower than on test *D*, but the curves were still within normal limits (figure 7*E*).

Patient T. B. (figure 8), a 13-month-old girl, was admitted on March 18, 1952, with early tuberculous meningitis. On admission the cerebrospinal fluid was clear, contained 111 leukocytes per cu. mm., 92 per cent of which were lymphocytes; 54 mg. of glucose per 100 ml.; 49 mg. of protein per 100 ml.; a streptomycin concentration of 2.5 γ per ml.; *M. tuberculosis* on culture. The first glucose test (*A*) was performed just before intrathecal therapy with streptomycin was begun. The patient was started on intramuscular streptomycin and oral isoniazid on March 20, 1952, and intrathecal streptomycin on March 21; the second glucose test (*B*) was performed on April 2, 1952, two days after streptomycin by the intrathecal route had been discontinued. The next day the cerebrospinal fluid contained 140 leukocytes per cu. mm., 80 per cent of which were lymphocytes; 66 mg. of glucose per 100 ml.; 118 mg. of protein per 100 ml.; and a streptomycin concentration of 10 γ per ml. Another glucose test (*C*) was performed on May 20, 1952, at which time the patient was convalescent from tuberculous meningitis, and her cerebrospinal fluid showed 4 leukocytes per cu. mm., 65 mg. of glucose per 100 ml., 50 mg. of protein per 100 ml. A pneumoencephalogram on July 23, 1952, showed the lateral ventricles to be within upper limits of normal in size.

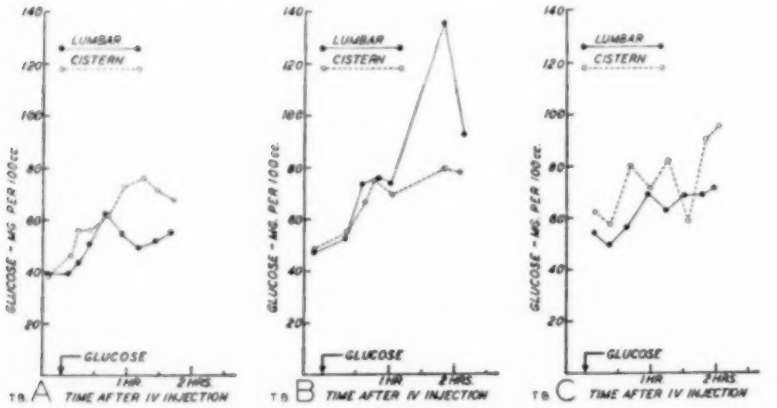

FIG. 8 (T. B.). Glucose tests in a 13-month-old girl with early tuberculous meningitis: (*A*) Before therapy; the relationship between cisternal and lumbar curves is normal. The cisternal rise is normal but the rise of the lumbar curve is below normal. (*B*) Fifteen days later and two days after streptomycin by the intrathecal route had been discontinued; the cisternal curve has not changed but there is a marked rise in the lumbar curve above the cisternal curve during the second half of the test. (*C*) Nine weeks later; the glucose test is within normal limits.

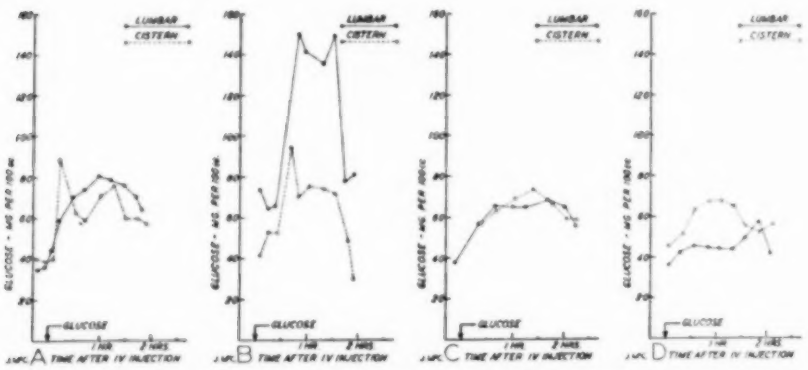

FIG. 9 (J. McC.). Glucose tests of a 4-year-old girl with moderately advanced tuberculous meningitis: (*A*) During the third week of treatment; shows an abnormal relationship between cisternal and lumbar curves except for a sharp early cisternal rise. Both curves show a normal rise. (*B*) Sixteen days later, seventy-two hours after a severe reaction to intrathecal streptomycin; the abnormal relationship between the cisternal and lumbar curves has become markedly accentuated. (*C*) Eight weeks after the beginning of treatment; the lumbar and cisternal curves are approximately at the same level. The rise of the lumbar curve is at the lower limits of normal. The rise of the cisternal curve cannot be evaluated because of delay in obtaining the first sample. (*D*) Five and a half months after the beginning of therapy and three weeks after discontinuing streptomycin, patient receiving isoniazid orally; glucose values in the cerebrospinal fluid are below normal. The relationship between cisternal and lumbar curves is normal but the rise of both curves is abnormally low.

The relationship between cisternal and lumbar curves was within normal limits (figure 8*A*). The cisternal rise above initial values was at the lower limits of normal. The lumbar rise was below normal.

The relationship between cisternal and lumbar glucose curves was reversed (figure 8*B*). The cisternal rise was similar to test *A* but the lumbar rise reached values much higher than the upper limits of normal.

The normal relationship between cisternal and lumbar glucose curves was restored (figure 8*C*). The cisternal rise above initial values was within normal limits, but the lumbar rise was still considerably below normal.

Patient J. McC. (figure 9), a 4-year-old girl, was admitted on November 23, 1951, with moderately advanced tuberculous meningitis. Treatment was started immediately with intramuscular and intrathecal streptomycin. On December 16, 1952, the first test (*A*) was performed. On that date the cerebrospinal fluid contained 66 leukocytes per cu. mm., 100 per cent of which were lymphocytes; 103 mg. of protein per 100 ml.; 24 mg. of glucose per 100 ml.; and a streptomycin concentration of 5 γ per ml. On December 21, 1951, a pneumoencephalogram was normal. On December 31, she had a reaction to intrathecal streptomycin which was characterized by gross clonic movements, dilated pupils, and absent knee jerks bilaterally. The cerebrospinal fluid on January 2, 1952, was bloody and contained 194 mg. of protein per 100 ml. and 39 mg. of glucose per 100 ml. The second glucose test (*B*) was done the following day and seventy-two hours after the reaction to intrathecal streptomycin. A repeat pneumoencephalogram two weeks later was normal. The third glucose test (*C*) was done on February 10, 1952, at the time of the sixty-fifth intrathecal injection. Cerebrospinal fluid at that time showed 64 leukocytes per cu. mm., 85 per cent of which were lymphocytes; 36 mg. of glucose per 100 ml.; 161 mg. of protein per 100 ml.; a streptomycin concentration of 10 γ per ml. On April 16, 1952, the intrathecal and intramuscular streptomycin was discontinued and oral isoniazid was started. The child became free from all symptoms and signs. The cerebrospinal fluid glucose, however, remained below 40 mg. per 100 ml. The fourth glucose test (*D*) was performed on May 7, 1952, at which time the cerebrospinal fluid contained 1 leukocyte per cu. mm., 39 mg. of glucose per 100 ml., and 59 mg. of protein per 100 ml. A pneumoencephalogram on March 27, 1952, was normal.

The normal relationship between the cisternal and lumbar curves was reversed except for one point in the cisternal curve that showed a sharp rise during the first fifteen minutes of the test (figure 9*A*). The rise of both cisternal and lumbar curves was within normal limits.

The abnormal relationship between cisternal and lumbar curves had become markedly accentuated on the second test (figure 9*B*). The rise in the lumbar curve was much greater than the upper normal limits of 60 mg. of glucose per 100 ml. above the initial values. The rise in the cisternal curve above the initial values was greater than on the previous test but was still within normal limits. The rapid profound drop in the cisternal glucose at the end of two hours was very striking.

The relationship between cisternal and lumbar curves was returning to normal (figure 9*C*). The lumbar rise was within the lower limits of normal. The cisternal rise could not be evaluated accurately because true initial values were not obtained.

The relationship between the cisternal and lumbar curves was normal (figure 9*D*) on the fourth test. The rise of both curves above initial values was definitely below normal.

Patient I. G. (figure 10), a 2-year-old boy, was admitted on May 21, 1951, with moderately advanced tuberculous meningitis. Treatment was started promptly with intramuscular and intrathecal streptomycin and Promizole. On August 29, 1951, it was decided to treat with intrathecal PPD because of increased drowsiness and a cerebrospinal fluid protein concentration of more than 300 mg. per 100 ml., which aroused suspicion of block. However, the streptomycin diffusion index was 1.0. A right frontal burr hole was made and treatment with PPD through the ventricular and lumbar routes was given until September 24, 1951. The patient became comatose and responded to PPD with fever and convulsions. On October 3, the cerebrospinal fluid showed 270 leukocytes, 100 per cent of which were lymphocytes; 60 mg. of glucose per 100 ml.; 375 mg. of protein per 100 ml.; a streptomycin concentration was less than 0.6 γ per ml. The first glucose test (*A*) was performed at this time. A pneumoencephalogram, five days later, was normal. Intrathecal streptomycin was discontinued on October 29, 1951. The patient came out of coma and improved rapidly. The second glucose test (*B*) was done on December 16. On that date the cerebrospinal fluid contained no leukocytes, 56 mg. of glucose per 100 ml., 62 mg. of protein per 100 ml., and a streptomycin concentration of 1.25 γ per ml. Intramuscular streptomycin was discontinued two weeks later. The patient continued to do well and the cerebrospinal fluid was normal at the time of the third test (*C*) on April 18, 1952.

This was one of the early tests, and fewer samples than usual were obtained. The normal relationship between lumbar and cisternal curves was reversed (figure 10*A*). The ventricular curve appeared to be just below the lumbar. The rise of the ventricular and lumbar curves above initial values was within normal limits. The cisternal rise was at the lowest limit of normal.

The appearance and rise of both curves were normal on the second test (figure 10*B*).

The relationship of the curves to each other and the rise above initial values were within normal limits on the third test (figure 10*C*).

Patient L. K. (figure 11), a girl two and a half years old, was admitted on August 25, 1951, with early tuberculous meningitis. The cerebrospinal fluid contained 40 leukocytes per cu. mm., 80 per cent of which were lymphocytes; 17 mg. of glucose per 100 ml.; 141 mg. of protein per 100 ml.; and a streptomycin concentration of 5 γ per ml. Treatment was begun immediately with streptomycin, intramuscularly and intrathecally, and Promizole. The patient improved very slowly and had persistent low cerebrospinal fluid glucose. A streptomycin diffusion index on September 27 was 1.0. A pneumoencephalogram on October 25, 1951, showed moderate hydrocephalus. The cerebrospinal fluid on November 5 was xanthochromic and contained 130 leukocytes, 100 per cent of which were lymphocytes; 32 mg. of glucose per 100 ml.; 190 mg. of protein per 100 ml.; and a streptomycin concentration of 40 γ per ml. The following day the first glucose test (*A*) was performed. The patient improved and became less irritable. A pneumoencephalogram on December 12 showed no change. A second glucose test (*B*) was performed two days later. On that date she had received 100 injections of streptomycin by the intrathecal route, and the cerebrospinal fluid showed 30 leukocytes, 100 per cent of which were lymphocytes; 42 mg. of glucose per 100 ml., 79 mg. of protein per 100 ml. One month later the intrathecal streptomycin was discontinued and the cerebrospinal fluid was normal. On February 26, 1952, the intramuscular streptomycin was discontinued. A third glucose test (*C*) was performed on February 6, 1952. A repeat pneumoencephalogram on May 2, 1952, showed no change in the degree of hydrocephalus. The cerebrospinal fluid findings were persistently normal. The fourth glucose test (*D*) was performed on July 1, 1952.

On the first test, the normal relationship between the cisternal and lumbar glucose curves was reversed (figure 11*A*). The rise above initial values should

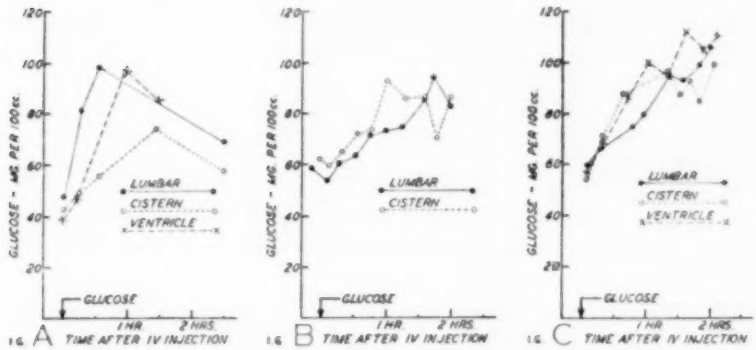

FIG. 10 (I. G.). Glucose test of a 3-year-old boy with moderately advanced tuberculous meningitis: (*A*) Nineteen weeks after beginning treatment and eleven days after completion of treatment with intrathecal PPD; patient was in coma at the time of the test. This is one of the early tests and fewer samples were obtained than in later tests. The normal relationship between the lumbar and cisternal curves is reversed. The rise of all curves is within normal limits. (*B*) Ten weeks later and seven weeks after intrathecal streptomycin was discontinued; the curves are normal. (*C*) Eleven months after the onset of the disease and four months after the last abnormal cerebrospinal fluid; the curves are within normal limits.

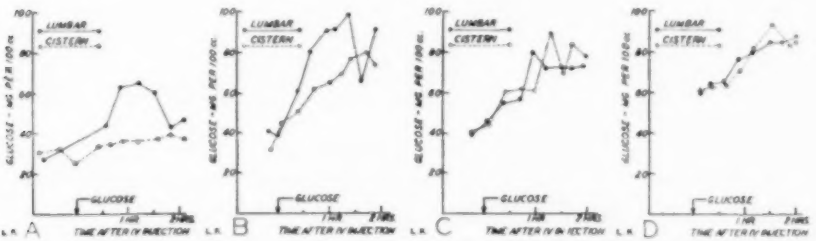

FIG. 11 (L. K.). Glucose tests in a girl two and a half years old with tuberculous meningitis and moderate hydrocephalus: (*A*) Eleven weeks after beginning treatment for meningitis; the normal relationship between the cisternal and lumbar curves is reversed. The rise of the lumbar curve is normal despite the fact that only half the usual dose of glucose was given. The cisternal curve fails to rise. (*B*) Seven weeks later while patient was still on intrathecal streptomycin; the abnormal relationship between the lumbar and cisternal curves persists. The rise of both curves is within normal limits. (*C*) Three weeks after discontinuing intrathecal streptomycin; at most points the relation of the cisternal to the lumbar curve is normal. The rise of both curves is within normal limits. (*D*) Ten months after beginning therapy for meningitis and four months after therapy with streptomycin had been discontinued; the cisternal and lumbar curves are at about the same level. The rise of the cisternal curve is at the lowest limits of normal and the lumbar rise is just below normal.

not be compared with that of other tests because only half the usual amount of glucose was given intravenously. In spite of this the lumbar rise was within normal limits. The cisternal rise was minimal.

The abnormal relationship between lumbar and cisternal curves persisted on the second test (figure 11*B*). The rise in the cisternal and lumbar curves was within normal limits.

The relationship between the cisternal and lumbar curves was returning to normal on the third test (figure 11*C*). The rise of both curves above initial values was within normal limits.

The relationship between the cisternal and lumbar curves had not changed by the time of the fourth test (figure 11*D*). The rise of the cisternal curve above initial values was at the lower limits of normal and the lumbar rise was just below normal.

Patient S. S. (figure 12), a girl one and a half years old, was admitted on April 4, 1951, with miliary tuberculosis. While on treatment with intramuscular streptomycin and Promizole she developed tuberculous meningitis manifested by eight cerebrospinal fluid cultures containing tubercle bacilli. Intrathecal streptomycin was started on May 2, 1951, and discontinued after forty injections. The cerebrospinal fluid was normal after June 1951, but the patient continued to show hyperactive deep tendon reflexes and bilateral ankle clonus. A glucose test was performed on January 31, 1951, at which time the cerebrospinal fluid contained no leukocytes, 61 mg. of glucose per 100 ml., 19 mg. of protein per 100 ml., and a streptomycin concentration of 0.6 γ per ml. A pneumoencephalogram four weeks later showed marked communicating hydrocephalus. Another glucose test (*B*) was performed on March 8, 1952. On May 12, 1952, bilateral frontal burr holes were made to decompress the ventricles. Six weeks later another glucose test (*C*) was performed. The patient's clinical condition had not changed and the frontal burr holes were bulging.

On the first test (figure 12*A*), both cisternal and lumbar curves were at approximately the same level and failed to rise significantly.

There was no change on the second test (figure 12*B*) except for a slight lumbar rise at the end of two hours.

On the third test (figure 12*C*), the ventricular, cisternal, and lumbar curves were at approximately the same level, the ventricular being slightly higher than the others; none of the curves showed a significant rise.

Patient B. Y. (figure 13),[7] a 10-month-old girl, was admitted to Babies Hospital in February, 1951, with moderately advanced tuberculous meningitis. Treatment was started immediately with streptomycin (intramuscularly and intrathecally), Promizole, and, subsequently, para-aminosalicylic acid. The cerebrospinal fluid culture was reported positive for tubercle bacilli. The patient became semicomatose and failed to improve. For this reason burr holes were done and PPD was given intrathecally from February to October, 1951. During May, 1951, a ventriculogram showed marked hydrocephalus. In November, 1951, a pneumoencephalogram again showed marked hydrocephalus. The cerebrospinal fluid was normal after October 25, 1951. The cerebrospinal fluid on December 26 contained 5 leukocytes, 57 mg. of glucose per 100 ml., 11 mg. of protein per 100 ml., chloride concentration of 123 mEq. per liter, pressure 210 mm. of water. The glucose test was done three days later.

The ventricular, cisternal, and lumbar curves were at approximately the same level and failed to rise except for a marked lumbar rise at one point, near the end of two hours (figure 13).

[7] This patient was tested at the Babies Hospital, Columbia Presbyterian Medical Center, New York, through the courtesy of Dr. Hattie Alexander.

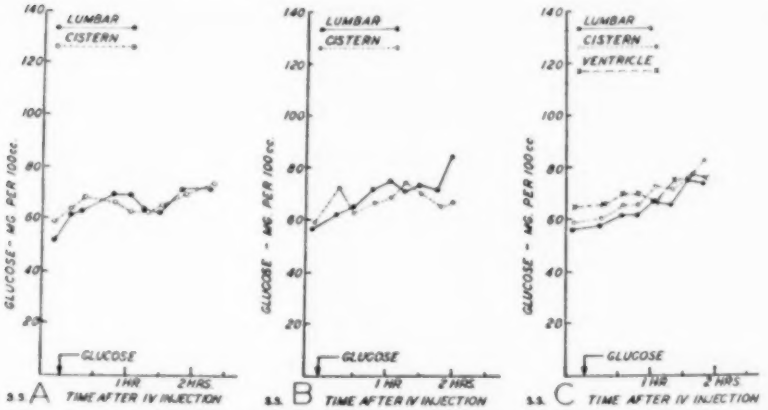

FIG. 12 (S. S.). Glucose tests in a girl one and a half years old with arrested tuberculous meningitis and marked hydrocephalus: (*A*), (*B*), and (*C*); the curves in all three tests show a minimal rise.

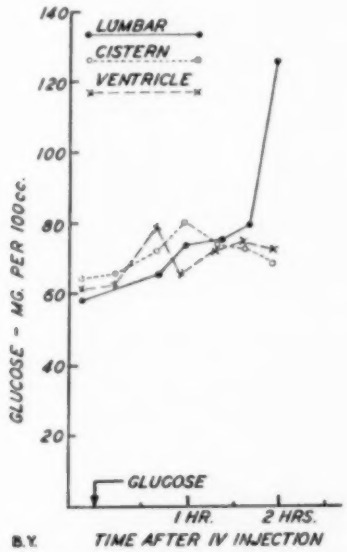

FIG. 13 (B. Y.). Glucose test in a 13-month-old girl with arrested tuberculous meningitis and marked hydrocephalus. The ventricular, cisternal, and lumbar curves are at approximately the same level and show a minimal rise except for a lumbar rise in one point at the end of two hours.

Patient B. K. (figure 14), an 8-month-old girl, was admitted on August 17, 1951, with a moderately advanced tuberculous meningitis. She was treated with streptomycin, intrathecally and intramuscularly, and Promizole orally. A roentgenogram of the skull on September 10, 1951, showed marked separation of the sutures. The head circumference was

increasing at the rate of 2.5 cm. in eighteen days. The streptomycin diffusion index was 0.3, indicating partial block. Methylene blue injected in the lumbar region failed to appear in the cistern. A right frontal burr hole was made on September 25 in order to treat through the ventricle, and subsequently all intrathecal streptomycin was given through this route. Tubercle bacilli were obtained on culture of the ventricular fluid. A glucose test was performed on October 4, 1951. On that date the ventricular fluid showed 1 leukocyte, 55 mg. of glucose per 100 ml., 170 mg. of protein per 100 ml. The patient developed decerebrate rigidity and died on October 11, 1951. Permission for autopsy was refused.

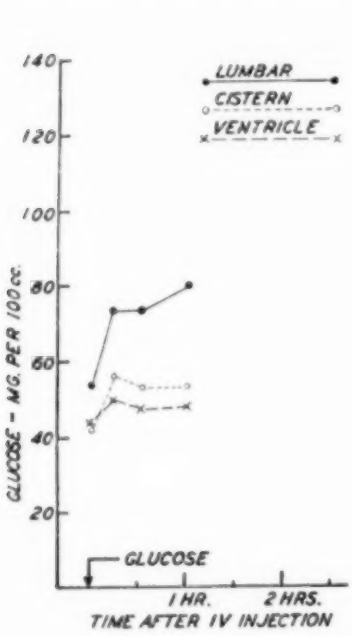

FIG. 14

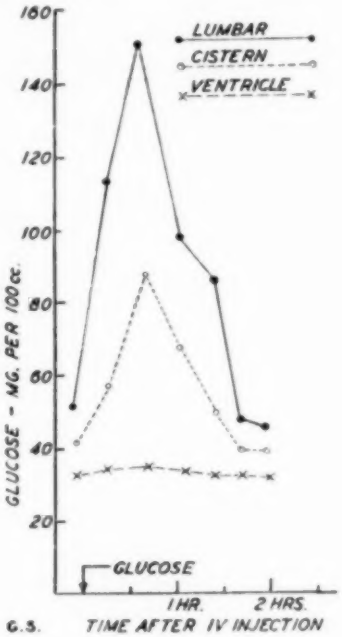

FIG. 15

FIG. 14 (B. K.). Glucose test in an 8-month-old girl with far advanced tuberculous meningitis, hydrocephalus, and block. The normal relationship between the lumbar and cisternal curves is reversed. The ventricular curve is abnormally placed below the cisternal curve. The rise of all curves is abnormally low; however, this patient received only half the usual amount of glucose.

FIG. 15 (G. S.). Glucose test in a 6-month-old boy with far advanced tuberculous meningitis, partial spinal block, and marked hydrocephalus. There is no rise of the ventricular glucose. The rise in the cisternal curve is within normal limits and the rise in the lumbar curve is very sharp and markedly above normal limits.

The normal relationship between the lumbar and cisternal glucose curves was reversed (figure 14). The ventricular curve was also abnormally placed below the cisternal curve. The rise in all curves was below normal; however, this patient received only half of the usual amount of glucose.

Patient G. S. (figure 15), a 6-month-old boy, was admitted on March 4, 1952, with far advanced tuberculous meningitis. He was treated with streptomycin, intrathecally and intramuscularly, and with Promizole and isoniazid orally. The intrathecal therapy was given alternately in the lumbar, cisternal, and ventricular regions because the diffusion

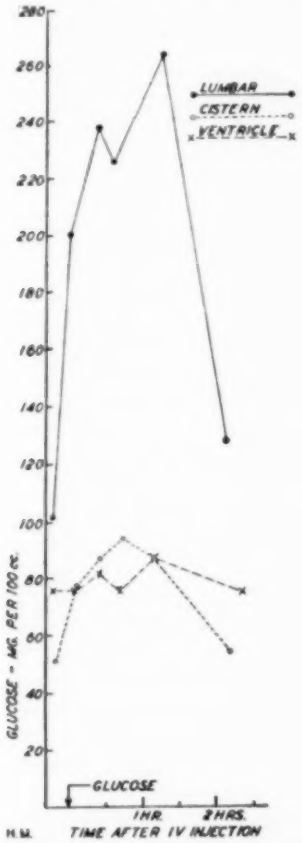

FIG. 16 (H. M.). Glucose test in a 2-year-old boy with far advanced tuberculous meningitis, complete spinal block, and marked hydrocephalus. The rise of the ventricular curve is minimal. The rise of the cisternal curve is within normal limits, whereas the lumbar shows a very marked, sharp rise.

index was 0.25, indicating partial block. On March 29, a pneumoencephalogram showed marked communicating hydrocephalus. A week later a glucose test was performed. On that day the cisternal fluid contained 38 mg. of glucose per 100 ml. and 486 mg. of protein per 100 ml.; the ventricular fluid contained 42 mg. of glucose per 100 ml. and 436 mg. of protein per 100 ml. The last lumbar puncture had been done three days previously, and the cerebrospinal fluid contained 1,500 leukocytes per cu. mm., of which 75 per cent were lymphocytes; 28 mg. of glucose per 100 ml.; 568 mg. of protein per 100 ml. The patient ran a downhill course and died on May 5, 1952. Autopsy permission was refused.

The normal relationship between the lumbar and cisternal curves was reversed and the ventricular curve was abnormally located, below the lumbar and cisternal curves (figure 15). The lumbar glucose curve showed a sharp rise, much greater than the normal rise. The cisternal rise was within normal limits and the ventricular glucose failed to increase at all.

Patient H. M. (figure 16), a 5-month-old boy, was admitted on January 28, 1950, with miliary tuberculosis. During treatment with intramuscular streptomycin and Promizole he developed tuberculous meningitis, and intrathecal streptomycin was added. The course was characterized by transient improvement followed by recrudescences. The patient had burr holes and was treated with PPD intrathecally from February 22 to September 25, 1951. On October 3, all treatment was discontinued because the outlook was hopeless. A glucose test was done on October 9, 1951. The patient died two days later, and autopsy showed marked hydrocephalus. Tuberculous exudate was present below the optic chiasma anteriorly, covering the pons, interpeduncular space, and the cisterna magna; more exudate was present in the spinal cord, blocking the regions above T4 and below T10. Another block was located in the aqueduct of Sylvius.

The normal relationship between the lumbar and cisternal curves was reversed (figure 16). The ventricular curve crossed the cisternal curve at about the same level. The lumbar glucose curve rose to extraordinarily high levels. The cisternal glucose curve showed a rise within normal limits, and the ventricular curve failed to show a significant rise.

DISCUSSION

A consistent pattern of changing concentrations of lumbar, cisternal, and ventricular glucose following intravenous injection of glucose has been noted in patients without meningitis and a similar one in patients with healed tuberculous meningitis. Observations on patients in various stages of tuberculous meningitis revealed different patterns from those established as normal.

It is probable that the passage of glucose from the blood into the cerebrospinal fluid reflects the permeability of the barrier for glucose and that changes in the passage of glucose into the cerebrospinal fluid represent changes in the permeability of the barrier except in hydrocephalus, where a dilution factor should be considered.

Apparently, in a child with tuberculous meningitis, with low cerebrospinal fluid glucose and who is not receiving intrathecal medication, a variety of abnormalities may be encountered. Four children had evidence of decreased permeability for glucose in both cisternal and lumbar regions. One child had marked increase in the permeability for glucose in the lumbar region and one had increased permeability in the cisternal region. None of these children had a glucose curve which was normal in all respects.

After therapy was started with intramuscular and intrathecal streptomycin, all patients showed a relative increase in the permeability for glucose in the lumbar region, so that the relationship between the lumbar and cisternal glucose curves was reversed. In 2 patients the relationship between the lumbar and cisternal curves returned to normal while they were still on intrathecal therapy.

Three children with normal ventricles on pneumoencephalography had persistently decreased permeability for glucose in the cisternal region during or after intrathecal therapy. In these children, the permeability for glucose in the lumbar region was also below normal in several curves, even when the lumbar curve rose above the cisternal.

The observation that the permeability of the meninges for passage of glucose may be altered in tuberculous meningitis suggests that this change in permeability is an important factor in producing the low cerebrospinal fluid glucose. Several patients who had low glucose values initially showed a return to low values within two hours even when there was a sharp rise of the glucose curve. This points to an abnormality of the blood-cerebrospinal fluid barrier as a causative factor in the low cerebrospinal fluid glucose since it is very unlikely that glucose could be metabolized by bacterial or central nervous system cells within a two-hour period. In one case of hemophilus influenza meningitis with low cerebrospinal fluid glucose values, a decreased permeability in the lumbar and cisternal regions was observed. Perhaps a similar mechanism of altered permeability may be a factor in producing low cerebrospinal fluid glucose values in purulent meningitis.

The role of intrathecal streptomycin in increasing the permeability for glucose in the lumbar region is not entirely clear. The reversal of the relationship between cisternal and lumbar glucose curves coincides with the administration of streptomycin in the lumbar region. A streptomycin-induced inflammation of the subarachnoid space has been demonstrated by Ramon Guerra (9) in rabbits injected with streptomycin in the lumbar region. The inflammation was most intense in the lumbar subarachnoid space and very mild in the cerebral subarachnoid space.

A marked increase in the permeability for glucose in the lumbar region was also observed in 3 patients who had not recently received streptomycin in the lumbar region at the time of the test and who had evidence of partial or complete spinal block. This observation was most clearly demonstrated in one of the 3 patients in whom the exudate producing spinal block was visualized at autopsy. A marked rise of the lumbar glucose curve suggestive of a spinal block was noted in a patient with normal streptomycin diffusion index and normal pneumoencephalogram, but with cerebrospinal fluid protein values consistently above 300 mg. per 100 ml. and low cerebrospinal fluid pressure. It is believed that this patient had an incomplete block that could not be detected by the usual means.

It seems probable that under abnormal conditions glucose can pass from the blood into any portion of the cerebrospinal fluid.

In his work, using heavy water and radioactive sodium in the normal man, Sweet and his associates (11) have concluded that the ingredients of the cerebrospinal fluid arise by two processes: one in which an isotonic solution derived from the blood slowly enters the ventricle; in the other, there is a rapid migration of sodium-poor water in opposing direction across the barrier. This explanation might offer the only other possible reason for the local increase in lumbar

glucose in the cases of the present study. The sudden artificial hyperglycemia might produce a sudden migration of water from the inflamed lumbar subarachnoid space into the blood, leaving a concentrated cerebrospinal fluid which would show an apparent increase in glucose. If such were true, other substances in the cerebrospinal fluid would increase at the same time. In a few cases, however, parallel determinations of the concentrations of proteins, urea, chlorides, and creatinine have shown no increase. For this reason, the present writers are inclined to the belief that a real increase in the permeability for glucose occurs locally.

When hydrocephalus developed in children with tuberculous meningitis, the increase in concentration of glucose in the ventricle was below normal in all cases. The cisternal glucose curve was below normal also except in those patients who had spinal block. The lumbar glucose curve was flat only in patients with hydrocephalus who had no spinal block and were not receiving intrathecal therapy. The flat curves associated with hydrocephalus might be due to a dilution of the glucose that entered the cerebrospinal fluid or to a decreased permeability of the blood-cerebrospinal fluid barrier. Perhaps both factors play a part. In children with congenital hydrocephalus (10), glucose curves were determined which were similar to the curves in patients with healed meningitis and hydrocephalus.

SUMMARY

The results of two-hour curves showing the passage of glucose from the blood into the cerebrospinal fluid following intravenous injection of glucose are reported.

Five children without meningitis and 6 children with healed tuberculous meningitis showed similar curves, characterized by an increase in the glucose concentration of the lumbar and cisternal fluid of 30 to 60 mg. per 100 ml. above initial values; the maximum increase occurred between forty-five and one hundred and twenty minutes. The cisternal values were always slightly higher than the lumbar values.

Abnormal curves were obtained in children with active tuberculous meningitis and in children with hydrocephalus resulting from tuberculous meningitis. Abnormal curves are reported in 15 patients. The main abnormalities consisted of:

(*1*) Decreased permeability during the acute phase in several children

(*2*) Increased permeability in the lumbar region so that the lumbar curve rose above the cisternal in patients under treatment with intrathecal streptomycin or in patients with spinal block

(*3*) Flat ventricular curves in patients with hydrocephalus

The lumbar and cisternal curves in these patients were conditioned by the presence of block or by the administration of the streptomycin through the lumbar subarachnoid space. With improvement in the tuberculous meningitis, the abnormalities of the glucose curves disappeared.

It is concluded that: Marked alterations in the permeability of the meninges for glucose occur during the course of tuberculous meningitis; under abnormal conditions glucose can pass from the blood into the cerebrospinal fluid locally into any portion of the subarachnoid space; and that these changes may account for the low glucose values obtained in the cerebrospinal fluid in tuberculous meningitis.

SUMARIO

Observaciones del Efecto de la Hiperglucemia Inducida sobre el Contenido de Glucosa del Líquido Cefalorraquídeo en los Enfermos de Meningitis Tuberculosa

Preséntanse los resultados de curvas de dos horas que muestran el paso de la glucosa, de la sangre al líquido cafalorraquídeo, a continuación de la inyección endovenosa de la misma.

Cinco niños sin meningitis y 6 con meningitis tuberculosis cicatrizada revelaron curvas semejantes, caracterizadas por aumento de la concentración de glucosa en los líquidos lumbar y cisternal, de 30 a 60 mg. por 100 ml. por encima de los valores iniciales; el aumento máximo tuvo lugar entre cuarenta y cinco y ciento veinte minutos. Las cifras cisternales fueron siempre un poco más altas que las lumbares.

Obtuviéronse curvas anormales en los niños que tenían meningitis tuberculosa activa y en los que tenían hidrocefalia debida a meningitis tuberculosa. Preséntanse curvas anómalas para 15 enfermos. Las anomalías consistieron en:

(1) Hipopermeabilidad durante la fase aguda en varios niños.

(2) Hiperpermeabilidad en la región lumbar, de modo que la curva lumbar se elevó sobre la cisternal en los enfermos bajo tratamiento intratecal con estreptomicina o que tenían bloqueo raquídeo.

(3) Curvas ventriculares planas en los hidrocefálicos.

En esos enfermos, las curvas lumbares y cisternales estaban regidas por la presencia de bloqueo o por la administración de la estreptomicina a través del espacio subaracnoideo lumbar. Al mejorar la meningitis tuberculosa, desaparecieron las anomalías de las curvas de la glucosa.

Dedúcese que, durante la evolución de la meningitis tuberculosa, tienen lugar notables alteraciones en la permeabilidad de las meninges para la glucosa; en circunstancias normales la glucosa puede pasar localmente de la sangre al líquido cefalorraquídeo en cualquiera porción del espacio subaracnoideo, y que esas alteraciones pueden explicar las bajas cifras de glucosa obtenidas en el líquido cafalorraquídeo en la meningitis tuberculosa.

RESUME

Observations sur l'action de l'hyperglycémie provoquée sur la teneur en glucose du liquide céphalo-rachidien des malades atteints de méningite tuberculeuse

Les résultats de la courbe de deux heures montrant le passage du glucose dans le liquide céphalo-rachidien à la suite de l'injection intraveineuse sont exposés.

Des courbes similaires ont été observées chez 5 enfants indemnes de méningite et chez 6 enfants après guérison d'une méningite tuberculeuse. Ces courbes sont caractérisées par un accroisement de la concentration du glucose dans le liquide céphalo-rachidien de la région lombaire et du confluent postérieur, qui dépasse la valeur normale de 30 à 60 mg. pour 1000 ml.; l'augmentation maxima s'est manifestée entre quarante-cinq et cent-vingt minutes. L'hyperglycorachie était toujours plus élevée au niveau du confluent supérieur.

Chez l'enfant, des courbes anormales ont été obtenues dans la méningite tuberculeuse évolutive et dans l'hydrocéphalie consécutive à la méningite tuberculeuse. Des courbes anormales ont été observées chez 15 malades. Les anomalies principales consistent en:

1°—Réduction de la perméabilité dans la phase aiguë chez plusieurs enfants.

2°—Augmentation de la perméabilité au niveau de la région lombaire, de telle manière que la courbe lombaire a dépassé celle du confluent postérieur chez les sujets en cours de traitement par la streptomycine sous-arachnoïdienne ou porteurs d'un bloc intra-rachidien.

3°—Courbe ventriculaire plate chez les malades atteints d'hydrocéphalie.

Chez ces malades, la courbe lombaire et celle du confluent postérieur étaient déterminées par l'existence d'un bloc ou par l'administration de la streptomycine dans l'espace sous-arachnoïdien lombaire. Les anomalies des courbes du glucose disparaissaient au fur et à mesure que la méningite tuberculeuse s'améliorait.

En conclusion, il se produit des altérations marquées dans la perméabilité des méninges pour le glucose au cours de la méningite tuberculeuse; dans les conditions normales, le glucose peut passer localement du sang dans le liquide céphalo-rachidien dans n'importe quel segment de l'espace sous-arachnoïdien; ces modifications peuvant expliquer les valeurs basses de la concentration en glucose du liquide céphalo-rachidien dans la méningite tuberculeuse.

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PULMONARY FUNCTION IN PREGNANCY[1, 2, 3]

II. Comparison of the Effects of Pneumoperitoneum and Pregnancy in Young Women with Functionally Normal Lungs and Serial Observations During Pregnancy and Postpartum Pneumoperitoneum

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INTRODUCTION

Pulmonary tuberculosis sometimes regresses during the later months of pregnancy. The amelioration of symptoms and improvement in roentgenographic or bacteriologic status have been ascribed to the elevation of the diaphragm and consequent relaxation of the lungs (1). These findings led clinicians to employ artificial pneumoperitoneum to imitate the increased intra-abdominal pressure of pregnancy (2, 3). Others adopted this form of collapse therapy because improvement of pulmonary lesions was noted when pneumoperitoneum was induced accidentally or when it was used for the treatment of intestinal and peritoneal tuberculosis (4, 5).

In subsequent years artificial pneumoperitoneum has found wide acceptance throughout the world. It has been found useful in bilateral and disseminated disease and numbers among its advantages complete reversibility, a low incidence of complications, and small encroachment on breathing reserve. Evaluation of its clinical effectiveness has been difficult because its extensive use coincided with the discovery of streptomycin. An estimate of the popularity of this procedure can be gained from the fact that, during the past five years, more than 50,000 intraperitoneal injections of air have been made at the Sanatorium Division of the Boston City Hospital and the associated public health clinics. These have been done only on patients living within the city limits of Boston and unable to afford private care.

The Physiologic Effects of Pneumoperitoneum

In view of the widespread use of pneumoperitoneum, the scarcity of data concerning its effect on the respiratory apparatus is surprising. Again, as with observations on the

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effect of pregnancy, functional changes after induction of pneumoperitoneum have, with one exception, been studied only by measuring the vital capacity and maximum breathing capacity (5–7).

Banyai found the average vital capacity decreased by 15 per cent, and three-fourths of his patients showed a diminution of this volume after the first treatment (5). The subdivisions of the vital capacity were recorded spirographically by Leiner and Abramowitz (6). Whereas the total vital capacity remained unchanged in most of their sixteen patients, an increase of the inspiratory capacity and a decrease of the expiratory reserve were regularly observed. The maximum breathing capacity showed little change. A definite reduction of the vital capacity was observed by Steinlin when treatment was begun after one leaf of the diaphragm had been paralyzed (7). In these circumstances, the changes in the maximum breathing capacity were more variable. Diminution of hyperventilation and of the oxygen deficit was attributed to the stabilizing effect of pneumoperitoneum on paradoxical motions of the diaphragm.

The observations of the present writers of the entire lung volume profile before and after induction of pneumoperitoneum were limited to patients with severe pulmonary emphysema (8). Measurements of the functional residual capacity during pneumoperitoneum for pulmonary tuberculosis were reported by Wright and associates (9). Tabulated percentage changes of the functional residual capacity due to pneumoperitoneum in 17 patients indicated a 30 per cent reduction of this volume in the upright position and a 20 per cent reduction when measurements were made in recumbency. The diminution of the functional residual capacity attributable to pneumoperitoneum was comparable to that observed on changing from the upright to the supine position. The joint use of pneumoperitoneum and recumbency was most effective since this reduced the functional residual capacity by 46 per cent. Alterations of the maximum breathing capacity were variable, but there was a decrease in that of the majority of patients. Both Leiner and Abramowitz and Wright and associates concluded that penumoperitoneum interferes very little with ventilatory function and can be used freely in patients with a low pulmonary reserve.

The effects of pneumoperitoneum in relation to the extent of existing tuberculous disease have not been reported, and the severity of the pulmonary process was not mentioned in the foregoing publications. A comparison of the effects of pneumoperitoneum and pregnancy on pulmonary function has not been made.

Experiences with pneumoperitoneum have led the present writers to believe that the resultant alterations of pulmonary function are dependent upon the extent and nature of the pulmonary process, the presence of pleural complications, the degree of mobility of the diaphragm, the age of the patient, and the presence of complicating pulmonary emphysema.

The first part of the present report is concerned with a study of the effects of artificial pneumoperitoneum on pulmonary function in two groups of young women. Patients in the first group were thought to have functionally normal lungs, while those in the second group had demonstrable functional impairment. The results of these studies will be compared with observations made on 19 normal women who were examined by identical methods at intervals during and after pregnancy (1).

Postpartum Pneumoperitoneum

Exacerbations of pulmonary tuberculosis, seen immediately after delivery, have been a source of grave concern. Clinical deterioration in the early postpartum period has been

variously ascribed to hormonal imbalance, blood loss, changes in calcium metabolism, and the effort of labor. More frequently, it has been attributed to the sudden descent of the diaphragm, which resulted in increased negativity of the intrapleural pressures and greater tension on the lungs. It has been shown that pregnant patients under treatment with artificial pneumothorax require larger and more frequent refills immediately after delivery (10). The application of abdominal binders or sandbags has proved ineffective in preventing the sudden positional changes of the diaphragm (11). The suggested use of bilateral phrenemphraxis after delivery (12) has understandably found little favor.

The prophylactic induction of artificial pneumoperitoneum immediately after delivery to maintain the high position of the diaphragm was first suggested by Vajda (13) and Zwirner (14). Barnes first reported a case in which intraperitoneal injection of air was used to prevent sudden distention of the lungs in the postpartum period (11).

Induction of pneumoperitoneum a few days after delivery in patients with pulmonary tuberculosis is now accepted practice in many clinics (15, 16). The treatment is usually begun three to four days post partum, but in some cases a large volume of air has been introduced immediately after delivery (17) or even during labor (18). A number of cases have been reported in which pneumoperitoneum was induced during pregnancy (5, 16, 19). Refills were usually abandoned between the twenty-fifth and thirty-second weeks because of technical difficulties. The importance of reinstituting the air space immediately after delivery has been emphasized.

Physiologic or roentgenographic studies during pregnancy followed by pneumoperitoneum have not been reported. Since it is not known whether full-term pregnancy or pneumoperitoneum is more effective in producing pulmonary collapse, it has been questioned whether the pulmonary relaxation which results from full-term pregnancy can be duplicated by induction of pneumoperitoneum. The second portion of this paper deals with studies of 5 women in whom these dual observations were possible.

SELECTION OF SUBJECTS

All patients selected for this study were young women so that a strict comparison of data could be made with studies in this laboratory of normal pregnant subjects (1). All of the 23 patients were hospitalized for treatment of active pulmonary tuberculosis. The classification of the National Tuberculosis Association was not appropriate because it was not designed for functional evaluation. The pulmonary involvement was classified according to its effect on pulmonary function because it was found that the extent and severity of the disease importantly affect the physiologic alterations due to pneumoperitoneum.

The 9 patients in the first group had functionally normal lungs. Three patients had a single, small cavity, not more than one centimeter in diameter (figures 1*A*, 6*A*). Three others showed unilateral apical infiltration (figure 6*B*, *C*) and the infiltration was bilateral in the remaining three (figure 1*B*, *C*). The total area of infiltration on the antero-posterior chest film was no more than ten square centimeters in any case. All patients in this group responded well to treatment with strict bed rest, streptomycin twice weekly, para-aminosalicylic acid (PAS) daily, and pneumoperitoneum. Five patients had normal chest roentgenograms at the time of discharge (figure 1*C*). According to the NTA classification, three patients in this group had moderately advanced disease (figure 1*A*), and in the others the disease was minimal (figures 1*B*, *C*; 6*B*, *C*). Before treatment, fluoroscopic observation of

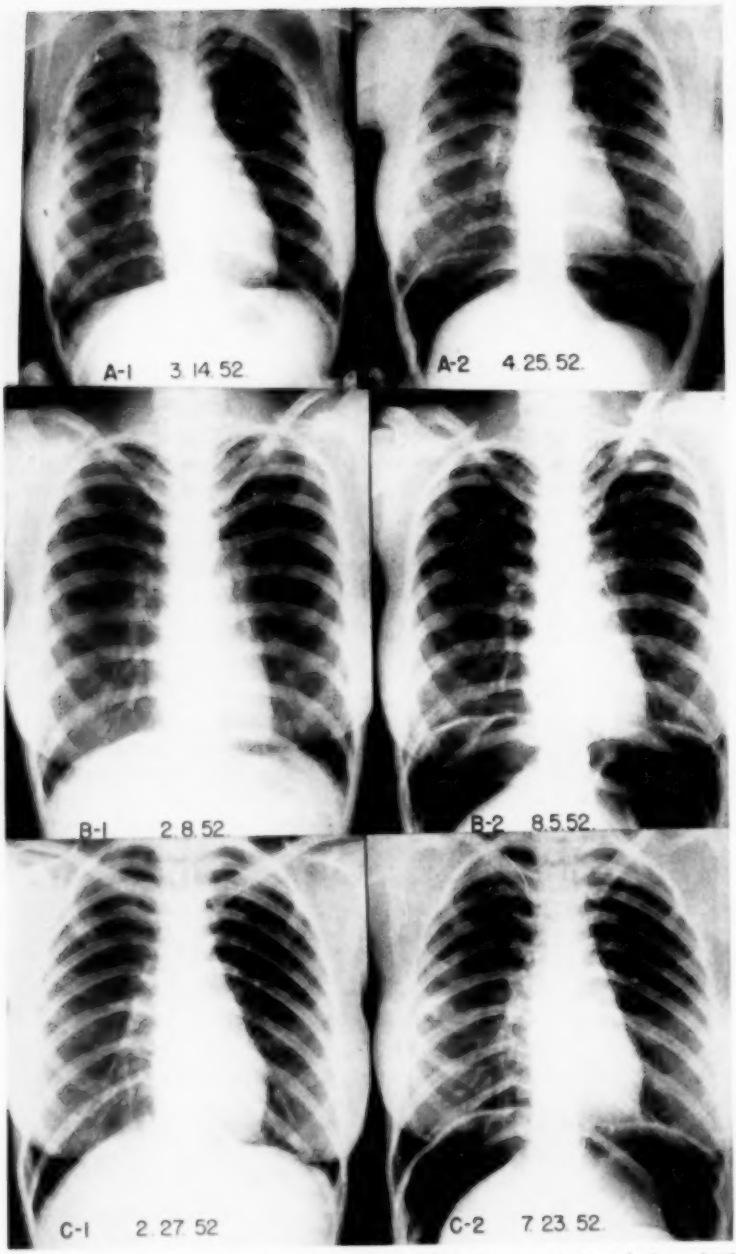

FIG. 1. Chest roentgenograms of 3 patients with pulmonary tuberculosis and "functionally normal lungs" before and after treatment with artificial pneumoperitoneum. Patient 8 of table 2 had a single cavity, 1 cm. in diameter, at the apex of the right lung (*A*). Patients 5 and 4 of table 2 had bilateral apical infiltration (*B* and *C*).

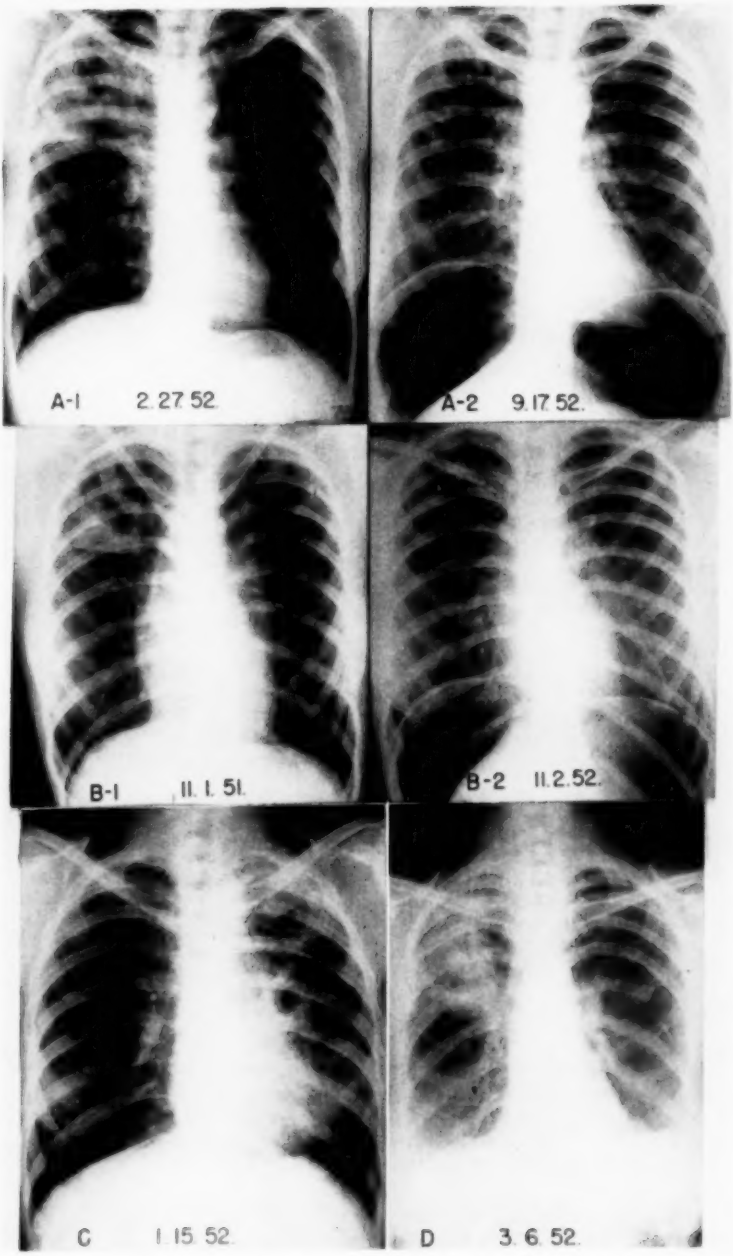

FIG. 2. Chest roentgenograms of 4 patients with pulmonary tuberculosis and "functionally impaired lungs." Patients in this group had more severe disease which involved an entire lobe. In Patients 1 and 2 (table 2) the parieties moved freely (*A* and *B*), but in one patient (Patient 3) fluoroscopy revealed asymmetrically reduced thoracic mobility (*C*) and in another (Patient 4) a fixed diaphragm was observed (*D*).

the chest revealed a normal range of motion of the diaphragm and thoracic cage in all patients of this group, and mediastinal shift or "air trapping" on forced expiration was not observed. In the subsequent discussion these patients will be referred to as those having lungs which are "functionally normal."

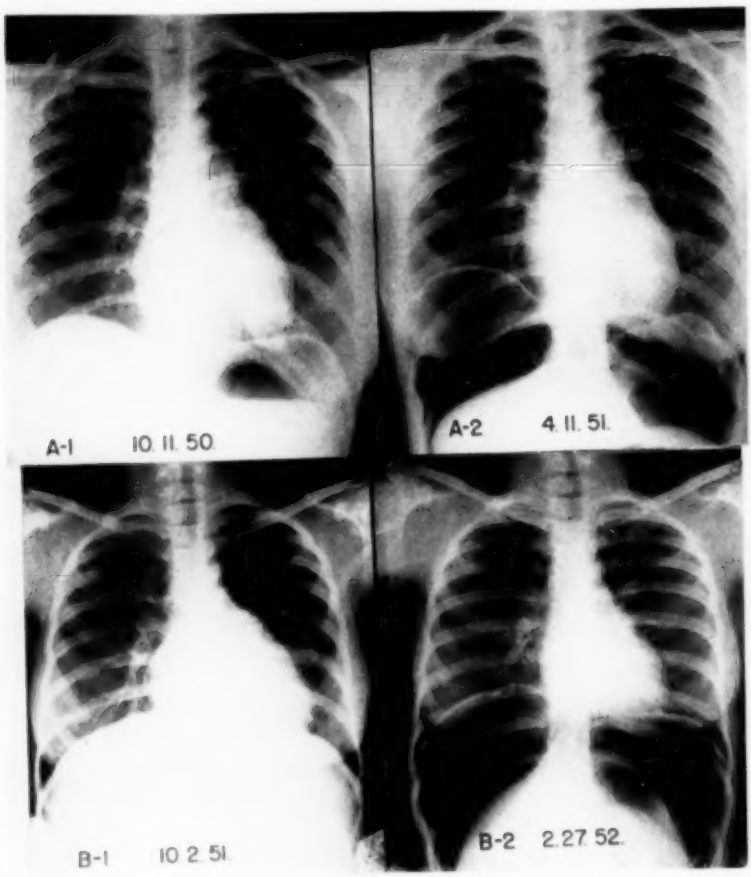

FIG. 3. Chest roentgenograms of 2 patients with minimal tuberculosis during the ninth month of pregnancy (*A-1* and *B-1*) and after postpartum induction of pneumoperitoneum (*A-2* and *B-2*). The elevated position of the diaphragm at the end of pregnancy is apparently well maintained by the air cushion.

The 9 patients in the second group will be referred to as those having lungs with "moderate functional impairment." All had severe disease involving one entire lobe (figure 2A, *B*, *C*) and 3 had some contralateral apical infiltration as well. *Patients were eliminated entirely from this study, however, if the history or roentgenograms suggested that the disease was of more than one year's duration, if there was marked fibrosis, or if function studies revealed pulmonary emphysema.* The patients in this second group had moderately or even far advanced disease

according to the NTA classification. Fluoroscopic observation of the chest revealed no mediastinal shift or "air trapping," but asymmetrically reduced thoracic mobility (figure 2*C*) or a relatively fixed diaphragm (figure 2*D*) was observed on three occasions.

At the Sanatorium Division, pneumoperitoneum is induced two to six days after delivery in patients whose disease has been stable or who have had a favorable course during pregnancy. Pneumoperitoneum is initiated during pregnancy if the disease progresses in spite of therapy and if surgery is contraindicated. If pneumoperitoneum is already present when pregnancy is recognized, refills are continued until the eighth month and then abandoned, to be reinstituted immediately after delivery. This therapeutic program provided an opportunity to study the physiologic effects of a variety of sequences and combinations of pregnancy and pneumoperitoneum.

The 5 patients who were studied during pregnancy and after postpartum pneumoperitoneum had only minimal tuberculosis (figure 3*A*, *B*) and no functional impairment. They were similar in all respects to the first group. All of the 5 patients were admitted within sixty days after the last menstrual period.

PLAN OF INVESTIGATION

Eighteen patients who were not pregnant were studied extensively one week before induction of pneumoperitoneum. This was usually two to six months after admission to the hospital and, in most cases, after one to three months' treatment with streptomycin and PAS. Studies were repeated when, in the opinion of the physician in charge, the pneumoperitoneum had been fully established. This was always at least six weeks after induction and at times it was two or even three months after the beginning of treatment.

Some of the later studies suggest that the maximal physiologic changes due to artificial pneumoperitoneum may not occur during the first three months after induction. As some of the patients were discharged early or operated upon (figure 2*C*), however, and as the criteria for selection were rigid, the accumulation of sufficient data at a later period was difficult and the early studies are therefore reported at this time. The physiologic changes associated with prolonged pneumoperitoneum treatment, as observed in patients of all ages, of both sexes, and with all degrees of disease, will be reported in another study (20).

The pregnant tuberculous patients were studied as soon after admission as the diagnosis of pregnancy was made. Observations were made in every case during the third month and the studies were repeated at monthly intervals and within two weeks of parturition. Further examinations were made one to six days post partum, immediately preceding the induction of pneumoperitoneum, six weeks later, and at the time of discharge, usually six months after delivery.

During treatment with pneumoperitoneum all observations were made forty-eight hours after the most recent air injection.

METHOD

The methods of evaluation of pulmonary function were the same as those used for the study of normal pregnant women (1). All measurements requiring voluntary maximal participation were made at least in triplicate and the best effort was recorded when repeated attempts checked within 5 per cent. A special effort was made to achieve optimal training and cooperation before the induction of

TABLE 1

COMPARISON OF CHANGES IN PULMONARY FUNCTION DUE TO PREGNANCY AND DUE TO PNEUMOPERITONEUM

DETERMINATION	PREGNANCY NORMAL VOLUNTEERS			PNEUMOPERITONEUM, NORMAL FUNCTION			PNEUMOPERITONEUM, IMPAIRED FUNCTION		
	Control	Term	Per Cent Change	Before	During	Per Cent Change	Before	During	Per Cent Change
Number of patients	19			9			9		
Age, years	22			23			31		
Body surface, m^2	1.64	1.76	+7	1.63	1.63	0	1.59	1.59	0
Respiratory rate	15	16	+10	12	12	0	16	16	0
Tidal volume, cc.	487	678	+39	585	548	−6	470	447	−5
Resting ventilation, L.	7.27	10.34	+42	7.02	6.57	−6	7.52	7.15	−5
Resting oxygen uptake, cc.	201	266	+32	237	225	−5	221	219	−1
Ventilatory equivalent, L/100 cc.	3.0	3.3	+10	2.5	2.4	−3	2.8	2.7	−4
Breathing reserve, per cent	92	89	−3	93	93	0	91	91	0
Walking ventilation, L/min.	14.7	19.1	+30	14.7	15.0	+2	16.5	16.3	−1
Walking index, per cent	15	21	+40	15	14	−7	19	20	+5
Maximum breathing capacity, L/min.	102	96	−6	95	106	+12	85	81	−5
Upright vital capacity, cc.	3,200	3,140	−2	3,174	3,158	−1	2,560	2,428	−5
Per cent of vital capacity, first second	84	82	—	78	80	—	79	78	—
Per cent of vital capacity, first two seconds	94	94	—	91	92	—	92	89	—
Per cent of vital capacity, first three seconds	98	97	—	97	95	—	96	95	—
Air velocity index	1.04	0.94	−10	1.00	1.12	+12	1.15	1.15	0
Inspiratory capacity, cc.	2,625	2,745	+5	2,619	2,859	+9	2,233	2,187	−2
Expiratory reserve, cc.	655	555	−15	631	519	−18	455	398	−11
Supine vital capacity, cc.	3,280	3,300	+1	3,250	3,378	+4	2,688	2,585	−4

TABLE 1—*Continued*

DETERMINATION	PREGNANCY NORMAL VOLUNTEERS			PNEUMOPERITONEUM, NORMAL FUNCTION			PNEUMOPERITONEUM, IMPAIRED FUNCTION		
	Control	Term	PerCent Change	Before	During	PerCent Change	Before	During	PerCent Change
Residual volume, cc.	965	770	−20	944	781	−17	941	876	−7
Functional residual capacity, cc.	1,620	1,325	−18	1,575	1,300	−17	1,396	1,274	−9
Total lung capacity, cc.	4,245	4,070	−4	4,194	4,159	−1	3,629	3,461	−5
R.V./T.L.C. × 100, per cent	23	19	−17	23	19	−17	26	25	−4
Intrapulmonary mixing index, per cent N_2	0.56	0.46	—	0.77	0.84	—	1.21	1.17	—

pneumoperitoneum. In 2 patients in whom continued improvement of voluntary efforts was observed, the initial air injections were deferred until repetition of studies produced a reliable baseline. The functional residual capacity and intrapulmonary mixing indices were determined in duplicate and the mean values were recorded.

OBSERVATIONS

The Effect of Pneumoperitoneum in Patients with "Functionally Normal" Lungs

The 9 women with "functionally normal" lungs were comparable, both in age and stature, to the 19 normal women observed during and after pregnancy (1) used here as a control group (table 1).

Studies before induction of pneumoperitoneum, summarized in table 1, proved that very limited pulmonary disease had no measurable effect upon function. The mean respiratory rate at rest was only 12, the basal oxygen uptake was normal, and the average ventilatory equivalent was 2.5. The minute ventilation, dyspnea index during standard exercise, and breathing reserve at rest were within normal limits. Studies of dynamic exchange of air showed an average maximum breathing capacity which was 100 per cent of the predicted value for the group, unimpaired timed vital capacities, and an air velocity index of 1.00. The lung volume compartments occupied the expected percentages of the total lung capacity. It was noteworthy that a comparison of the mean values of these compartments with similar observations in the normal volunteers showed a difference of less than 50 cc. in each instance (table 1, figure 4). The intrapulmonary mixing index was well below the upper limit of normal.

After pneumoperitoneum was fully established, the changes of resting ventilation and oxygen uptake were quite unlike those in the pregnancy group, whereas alterations in lung volumes were similar both in direction and in magnitude to those seen in pregnancy (table 1, figure 4).

The very small reduction of oxygen uptake was not statistically significant. A 16 per cent diminution of tidal volume and minute ventilation was observed in contrast to the findings of Leiner and Abramowitz of a slight temporary increase of these values (6). The standard exercise ventilation remained unchanged as would be expected with unchanged body weight.

An increase in maximum breathing capacity after pneumoperitoneum was a consistent finding in every person (table 2). A similar gain was observed in a number of male patients in another study in this laboratory. Statistical evalua-

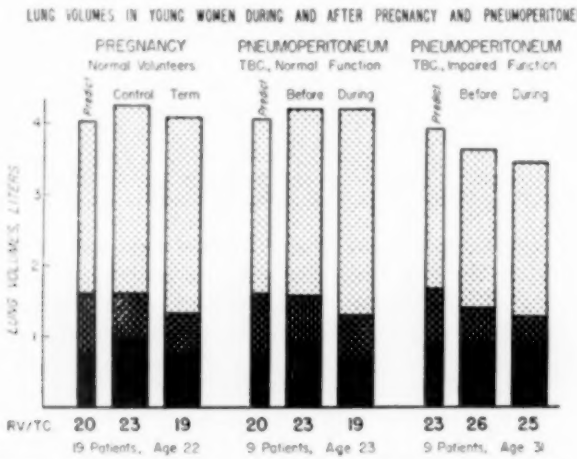

FIG. 4. Mean total lung capacities and their subdivisions in 19 normal subjects before pregnancy and at term, and in 18 young women with pulmonary tuberculosis (9 with "functionally normal" and 9 with "functionally impaired" lungs) before treatment and after induction of artificial pneumoperitoneum. "Normal" predicted values are indicated. In this and the subsequent illustration the subdivisions of the total lung capacity are identified as follows: black—*residual volume*; stippled black—*expiratory reserve*; stippled white—*inspiratory capacity*; the dark areas together represent *functional residual capacity*; and the stippled areas together, *vital capacity*.

tion showed that the chance that this increase was due to accidental sampling was less than 1 per cent and the mean difference was more than three times the standard error (table 2). This finding was unexpected because ordinarily a "treatment" is not regarded as being capable of improving an initially normal bodily function. The increase could not be attributed to an improvement in physical condition, for none of the patients in this group had experienced weight loss prior to admission and all were in good condition both before and after induction of pneumoperitoneum. The "training factor" was thought to have been eliminated by repeated testing during the control period. It appears that pneumoperitoneum was actually responsible for an increase of maximum breathing capacity in patients who had previously shown no measurable abnormality of pulmonary function.

TABLE 2

INDIVIDUAL VARIATION OF CHANGES IN LUNG VOLUMES DURING PREGNANCY AND DURING PNEUMOPERITONEUM TREATMENT

PATIENT NUMBER	MAXIMUM BREATHING CAPACITY, L./MIN. CHANGE			INSPIRATORY CAPACITY, CC. CHANGE			EXPIRATORY RESERVE, CC. CHANGE			RESIDUAL VOLUME, CC. CHANGE			FUNCTIONAL RESIDUAL CAPACITY, CC. CHANGE		
	Normal Function Pregnant	Normal Function Pneumoperitoneum	Impaired Function Pneumoperitoneum	Normal Function Pregnant	Normal Function Pneumoperitoneum	Impaired Function Pneumoperitoneum	Normal Function Pregnant	Normal Function Pneumoperitoneum	Impaired Function Pneumoperitoneum	Normal Function Pregnant	Normal Function Pneumoperitoneum	Impaired Function Pneumoperitoneum	Normal Function Pregnant	Normal Function Pneumoperitoneum	Impaired Function Pneumoperitoneum
1	−11	+13	+ 3	+290	+ 45	+230	−150	− 25	−130	−100	−110	−235	−250	−135	−365
2	+19	+ 6	+ 3	+230	+410	+ 45	−140	−140	−145	−180	−170	−115	−320	−310	−260
3	−14	+17	−11	−160	+655	−525	− 65	− 45	− 5	−195	−115	−360	−260	−160	−355
4	−17	+ 8	− 2	+435	+ 50	+ 30	−205	+185	+ 50	−375	−525	− 50	−580	−340	0
5	+ 6	+ 5	0	+285	+275	+175	−110	−220	−145	−115	+100	−185	−225	−120	−330
6	+ 8	+ 9	0	+ 95	+120	− 15	+ 80	−140	−215	−155	−375	+260	− 75	−515	+ 5
7	+ 3	+ 6	− 8	+330	+260	−180	+ 45	−345	− 25	−180	− 70	+ 60	−135	−385	+ 35
8	− 8	+17	−17	+500	+175	−110	−155	− 65	+220	− 40	− 85	+ 40	−195	−150	+260
9	−19	+16	− 8	− 20	+170	−105	−185	−195	− 90	−180	−160	0	−365	−355	− 90
10	−15			+100			− 65			−115			−180		
11	− 6			− 25			+ 20			−285			−265		
12	− 2			+235			− 80			−135			−215		
13	−12			−315			+ 25			−135			−110		
14	+ 3			−300			− 55			− 25			− 80		
15	− 6			+115			−190			+ 30			−160		
16	−14			−210			−200			−410			−210		
17	−16			+ 85			−115			−170			−285		
18	− 2			+380			−230			−180			−410		
19	−24			+200			−140			−430			−570		
Mean change	− 6	+11	− 4	+120	+240	− 51	−100	−112	− 52	−195	−163	− 65	−295	−275	−123
S. D.	±11	± 5	± 7	±221	±193	±222	± 97	±109	±144	±124	±161	±184	±148	±139	±217
S. E.	± 2.5	± 1.7	± 2.7	± 51	± 64	± 74	± 22	± 36	± 48	± 28	± 54	± 61	± 34	± 46	± 72
Mean, (t) / S. E.	2.4	6.5	1.5	2.3	3.8	0.7	4.5	3.1	1.1	7.0	3.0	1.1	8.7	6.0	1.7
Probability of t	5%	1%	20%	5%	1%	50%	1%	2%	30%	1%	2%	30%	1%	1%	20%

S. D. = standard deviation.
S. E. = standard error.

Pneumoperitoneum caused very definite and characteristic changes in the subdivisions of the total lung capacity. The mean inspiratory capacity was 9 per cent greater, the increase being observed in every subject (table 2). The expiratory reserve decreased 18 per cent, and the residual volume 17 per cent. These reductions, found consistently in individual patients, resulted in a 17 per cent smaller functional residual capacity after treatment. The residual volume, which had occupied 23 per cent of the total lung capacity before treatment, was 20 per cent of this volume after pneumoperitoneum treatment. The changes in the subdivisions were "compensatory" in that the mean total lung capacity remained within 35 cc. of the same volume in the control period (table 1, figure 4). The vital capacity remained essentially unchanged. The intrapulmonary mixing index remained within normal limits after induction of pneumoperitoneum; the slight increase of this value may have been due to a somewhat reduced minute ventilation (1).

The Effect of Pneumoperitoneum in Patients with Moderate Pulmonary Functional Impairment

The 9 patients in the group with moderate pulmonary functional impairment were, on the average, eight years older and of somewhat slighter stature than the patients with functionally normal lungs.

Before treatment, ventilatory studies at rest were within normal limits, but observations of dynamic exchange of air and of lung volume compartments revealed a definite pulmonary insufficiency of the restrictive type (table 1, figure 4).

Although resting minute ventilation was 7 per cent greater than in the patients with functionally normal lungs, alveolar ventilation was actually slightly less since the minute ventilation was augmented only by an increase in rate. Oxygen uptake was normal. The ventilatory equivalent was slightly elevated. These results were considered to be within normal limits. They were the same as those obtained in a group of normal women six months after pregnancy (table 1).

The average maximum breathing capacity of 85 liters per minute was 87 per cent of the predicted value for the group. This reduction was accompanied by a decrease of vital capacity of similar magnitude; hence, it was thought to be due to a diminution of stroke volume. There was no evidence of airway obstruction or loss of pulmonary elasticity: the air velocity index and the percentages of the vital capacity exhaled per unit of time were not diminished, and the pulmonary mid-position and the intrapulmonary mixing index were not elevated.

Following treatment with pneumoperitoneum, 5 per cent reductions of tidal volume and resting ventilation were similar in magnitude and direction to changes observed in patients with functionally normal lungs (table 1). The remaining observations showed a great deal more individual variation in response to pneumoperitoneum than was observed in the previous group. The maximum breathing capacity decreased in the majority of patients but the change of the mean was not statistically significant (table 2). A slight reduction of the upright and supine vital capacities was equally insignificant. The direction of changes of expiratory reserve, residual volume, and functional residual capacity was the same as in the first group but the magnitude of the changes was smaller (table 1)

and individual variation was much greater (table 2). The inspiratory capacity was reduced in contrast to its behavior in patients with functionally unimpaired lungs. This resulted in a reduction of the total lung capacity, which did not occur after pneumoperitoneum in patients with functionally normal lungs. A reduction of the lung volume in the pulmonary mid-position was achieved in most cases, the mean functional residual capacity being 9 per cent smaller than before treatment. While the elevated intrapulmonary mixing index of emphysema is reduced by pneumoperitoneum (8), the normal index in the present group remained unaffected.

Comparison of the ventilatory response of individual patients (table 2) with their chest roentgenograms (figure 2) yields a clue to the variability exhibited within this group.

The roentgenogram of a patient (Patient 3) with extensive destruction of the upper lobe of the left lung is shown in figure 2*C*. Changes of maximum breathing capacity and the subdivisions of total lung capacity are recorded as No. 3 in the "Impaired function, Pneumoperitoneum" columns of table 2. This patient's acute disease responded to bed rest, intensive chemotherapy, and pneumoperitoneum with contraction of the entire lung volume profile. The inspiratory capacity was reduced by 525 cc. and the functional residual capacity by 355 cc. These volumetric diminutions were accompanied by a 10-liter reduction of the maximum breathing capacity.

The roentgenogram of another patient (Patient 4) in this group revealed a flattened diaphragm and obliteration of the left costophrenic angle (figure 2*D*). An empyema on the left side had been drained many years before. Fluoroscopy demonstrated limited motion of the right leaf of the diaphragm and complete fixation of the left. The various subdivisions of the total lung capacity were not altered by pneumoperitoneum in this patient (table 2, Patient 4).

In contrast, Patients 1 and 2 in this same group (table 2), whose roentgenograms are shown in figures 2*A* and *B*, showed the response typical for patients with functionally normal lungs. The maximum breathing capacity and inspiratory capacity increased, and there was a satisfactory reduction of expiratory reserve and residual volumes. The disease was apparently of recent origin and as yet had not caused fixation of the lungs, thoracic cage, or diaphragm.

The varying physiologic responses to pneumoperitoneum of patients with more severe tuberculosis are probably due to differences in the type of disease and to the variable effect on pulmonary parenchymal tissue and its supporting structures. Comparison of pulmonary functional alterations with roentgenograms and fluoroscopic findings emphasizes the importance of considering the type and extent of disease when evaluating the therapeutic effect of artificial pneumoperitoneum.

Serial Observations During Pregnancy, After Delivery, and After Postpartum Pneumoperitoneum

Pulmonary function studies of 5 patients with minimal tuberculosis, studied throughout pregnancy, immediately after delivery, and six weeks and six months after induction of pneumoperitoneum, are shown in tables 3 and 4 and in figure

TABLE 3

RESTING AND EXERCISE VENTILATION DURING PREGNANCY, SOON AFTER DELIVERY, AND AFTER POSTPARTUM PNEUMOPERITONEUM
Mean Values, Five Patients

	WEIGHT KG.	BODY SURFACE M^2	RESTING								STANDARD WALKING EXERCISE		
			Tidal Volume, cc.	Respiratory Rate	Minute Ventilation		Minute Oxygen Uptake		Ventilatory Equivalent, L/100 cc.	Breathing Reserve, per cent	Minute Ventilation		Walking Dyspnea Index, per cent
					L.	$L./m^2B.S.$	cc.	$cc./m^2B.S.$			L.	$L./m^2B.S.$	
3 months pregnant	62	1.69	460	16	7.36	4.36	231	136	2.6	94	18	11	16
4 months pregnant	63	1.70	500	14	7.00	4.12	232	136	2.5	94	18	11	17
5 months pregnant	64	1.71	505	15	7.58	4.43	268	156	2.3	94	18	11	17
6 months pregnant	66	1.73	530	16	8.48	4.90	256	148	2.7	93	20	12	19
7 months pregnant	68	1.75	555	16	8.88	5.07	247	141	3.0	93	21	12	20
8 months pregnant	70	1.77	520	17	8.84	4.99	257	145	2.8	92	21	12	20
9 months pregnant	72	1.80	605	16	9.68	5.38	277	154	2.9	91	22	12	21
1 to 6 days post partum	64	1.71	515	17	8.76	5.12	240	140	3.0	91	20	12	22
6 weeks' pneumoperitoneum	64	1.71	480	16	7.68	4.49	211	129	2.9	94	18	11	18
6 months' pneumoperitoneum	64	1.71	410	15	6.15	3.60	235	137	2.2	94	17	10	17

TABLE 4

MAXIMAL VENTILATION, LUNG VOLUMES, AND INTRAPULMONARY MIXING DURING PREGNANCY, SOON AFTER DELIVERY, AND AFTER POSTPARTUM PNEUMOPERITONEUM

Mean Values, Five Patients

CONDITION	MBC L./MIN.	MBC PER CENT OF PREDICTED	UPRIGHT VC, L.	UPRIGHT VC, PER CENT OF PREDICTED	AIR VELOCITY INDEX	INSPIRATORY CAPACITY, PER CENT OF PREDICTED	EXPIRATORY RESERVE, PER CENT OF PREDICTED	VITAL CAPACITY, PER CENT OF PREDICTED	FUNCTIONAL RESIDUAL CAPACITY, PER CENT PREDICTED	RESIDUAL VOLUME, PER CENT OF PREDICTED	TOTAL LUNG CAPACITY, PER CENT PREDICTED	R.V./T.L.C. × 100, PER CENT	PULMONARY MIXING INDEX, PER CENT N_2
3 months pregnant	113	110	3.30	103	1.07	102	96	101	107	117	104	23	0.63
4 months pregnant	112	109	3.34	104	1.05	103	99	102	106	113	104	22	0.65
5 months pregnant	111	107	3.30	103	1.04	104	99	103	99	100	102	20	0.61
6 months pregnant	112	106	3.35	104	1.02	111	92	106	99	107	106	19	0.55
7 months pregnant	111	103	3.37	105	0.98	119	86	111	94	102	109	19	0.42
8 months pregnant	109	101	3.30	103	0.98	118	79	108	92	104	107	19	0.50
9 months pregnant	109	100	3.29	102	0.98	115	84	108	87	90	104	17	0.61
2 to 6 days post partum	97	94	3.25	101	0.93	104	81	99	97	112	101	22	0.84
6 weeks' pneumoperitoneum	102	98	3.21	100	0.98	113	77	104	79	81	100	16	0.86
6 months' pneumoperitoneum	105	102	3.13	97	1.05	111	68	100	75	83	97	17	0.69

MBC = maximum breathing capacity.
VC = vital capacity.

5. They had "functionally normal" lungs, and their chest roentgenograms showed areas of infiltration covering no more than ten square centimeters (figure 3).

The pulmonary functional alterations due to pregnancy followed the pattern previously observed in normal volunteers (1). A tendency toward hyperventilation could be detected as early as the third month if the data, obtained six months after pregnancy, were used as control values. At term, a 42 per cent increase of minute ventilation was accounted for by an increase in tidal volume, as in the normal volunteers. This efficient adjustment to increased ventilatory demand was thought to indicate adequate respiratory reserve. Pregnant women

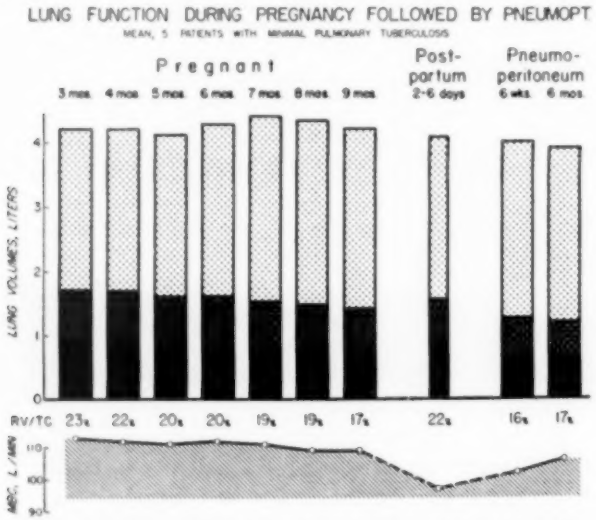
LUNG FUNCTION DURING PREGNANCY FOLLOWED BY PNEUMOPT
MEAN, 5 PATIENTS WITH MINIMAL PULMONARY TUBERCULOSIS
Pregnant
Post-partum
Pneumo-peritoneum
3 mos. 4 mos. 5 mos. 6 mos. 7 mos. 8 mos. 9 mos.
2-6 days
6 wks. 6 mos.
LUNG VOLUMES, LITERS
RV/TC
23% 22% 20% 20% 19% 19% 17% 22% 16% 17%
MBC, L./MIN.

FIG. 5. Mean lung volumes and maximum breathing capacities of 5 patients with minimal pulmonary tuberculosis. Observations were made throughout pregnancy, immediately after delivery, and at intervals after postpartum pneumoperitoneum treatment. The code is the same as in figure 4.

with pulmonary insufficiency meet the increased demand by increasing the respiratory rate (22). Again, oxygen uptake did not keep pace with ventilation. This resulted in a rising ventilatory equivalent. Exercise ventilation increased in proportion to body surface; the walking dyspnea index never rose above 21 per cent. The initial mean maximum breathing capacity of 113 per cent of predicted was the highest value recorded for any group in this series. Little change of this value was observed during pregnancy. The subdivisions of the total lung capacity are expressed as per cent of predicted in table 4 and as absolute volumes in figure 5. As in normal subjects, alterations in the various lung volumes did not occur until the second half of pregnancy. From the fifth month on, the expiratory reserve and the residual volume diminished progressively and, at term, the patients breathed with relatively deflated lungs. This did not affect the total lung

capacity because of a compensatory increase of the inspiratory capacity. The vital capacity in the upright position diminished very slightly, as it did in the normal subjects. In the recumbent position, however, an increase of this volume was observed with a peak performance during the seventh month.

Early postpartum studies showed a greater deviation from control values than had been noted in normal volunteers because some tests were made as early as one or two days after delivery while patients were still weak. In the nontuberculous mothers, observations were not made until the second postpartum week. The anticipated reduction of ventilation had not yet occurred and there was marked hyperventilation, as indicated by the ventilatory equivalent. The maximum breathing capacity was severely reduced. The residual volume showed the expected rapid increase. Obviously, studies at this time could not serve as control values, either for the preceding observations during pregnancy or for the following observations during pneumoperitoneum treatment. They did suggest, however, that any beneficial "collapse" of the lungs due to pregnancy is lost almost immediately after delivery.

Six weeks after induction of pneumoperitoneum, hyperventilation was still a prominent factor but the oxygen uptake had returned to basal values. Four to six months after pneumoperitoneum treatment, the lowest ventilatory requirements were observed both at rest and during exercise. The maximum breathing capacity improved slightly but it did not again reach the level observed during early pregnancy. Soon after treatment was started the vital capacity remained unaffected. Later, there was a small diminution, which was not observed in the previous two groups of patients treated with pneumoperitoneum, probably because no late follow-up studies were made.

The subdivisions of the total lung capacity, which had returned to "normal" levels immediately after delivery, once more showed the changes which are apparently characteristic of increased intra-abdominal pressure due to pregnancy or pneumoperitoneum. The inspiratory capacity was 9 per cent greater than during early pregnancy and 7 per cent greater than it was immediately after delivery. The expiratory reserve and residual volumes promptly decreased after induction of pneumoperitoneum, and six weeks later these volumes were smaller than during full-term pregnancy. At this time the decrease of the lung volume in the pulmonary mid-position was comparable to the reduction seen in the previous two groups of patients treated with pneumoperitoneum. Four to six months later, there was a further decrease of the functional residual capacity which was not accompanied by a compensatory increase of inspiratory capacity.

These observations suggest that the maximal collapse due to pneumoperitoneum treatment may not be attained until several months after treatment. Long-range studies of the effect of pneumoperitoneum in other patients with tuberculosis or with pulmonary emphysema appear to confirm this impression (9, 20).

This comparison of the effects of pregnancy and of pneumoperitoneum on pulmonary volumes in the same subjects suggests that the responses are similar. These observations further show that the pulmonary "collapse" due to full-term pregnancy can be maintained quantitatively by induction of pneumoperitoneum

in the postpartum period. Indeed, the diminution of lung volume at the point of forced expiration and at the end of quiet expiration was somewhat greater during pneumoperitoneum treatment than during pregnancy at term. However, the residual volume and functional residual capacity promptly returned to their original value after delivery. Therefore, if prolonged distension of the lungs is to be avoided, a fairly large pneumoperitoneum should be induced as soon as technically feasible after childbirth.

DISCUSSION

The effects of pregnancy and of pneumoperitoneum on oxygen uptake and ventilation were entirely different and the individual responses of subjects in either group were quite uniform. No further analysis was necessary to show a significant difference. Neither pneumoperitoneum nor pregnancy had a significant effect on ventilatory capacity. Dyspnea is a prominent complaint during pregnancy, however, because the ventilatory work is markedly increased. This increase is in part due to a measurably greater need for oxygen and partly due to less tangible physiochemical and neurogenic stimuli (1). Because these factors are absent during pneumoperitoneum treatment, dyspnea is a rare complaint. None of the 18 patients in the present study were troubled with shortness of breath during treatment. Although physical activity is voluntarily limited during hospitalization, it is known that when the patients leave the hospital they resume their usual daily tasks without discomfort. Dyspnea as a result of pneumoperitoneum is rare even in patients with severe pulmonary insufficiency. On the contrary, if long-standing disease is complicated by "compensatory" emphysema, dyspnea is often lessened by intraperitoneal injection of air (21). Dyspnea occasionally occurs when pneumoperitoneum is used in conjunction with phrenic paralysis. Unlike pneumoperitoneum alone, this combined treatment also often causes a marked reduction of the lung volumes and of the maximum breathing capacity (20).

A more detailed analysis of the changes in the subdivisions of the total lung capacity seemed worth while because the "collapse" effect is defined by these changes. The magnitude of the changes was often small, and, in certain instances, there was individual variation of direction as well. Alterations of maximum breathing capacity and of the subdivisions of total lung capacity are shown for normal subjects during pregnancy and for tuberculous patients with functionally normal and functionally impaired lungs who were treated by pneumoperitoneum (table 2). The statistical analysis was performed according to the method used in the first paper of this series (1). The standard deviation about the mean (S.D.), the standard error of the mean (S.E.), and Fisher's t value were calculated. The probability of t expressed the chances, in per cent, of finding such a difference due to accidental sampling.

The changes of maximum breathing capacity were never large, but a 6-liter decrease during pregnancy and an 11-liter increase with pneumoperitoneum in patients with functionally normal lungs were significant at the 5 and 1 per cent levels, respectively. The slight reduction of maximum breathing capacity due to

pneumoperitoneum in patients with functionally impaired lungs was not significant; there was a 20 per cent chance that this mean change was due to random sampling.

The difference in levels of significance of the changes in the subdivisions of the total lung capacity were striking (table 2). The alterations due to pregnancy in normal subjects and due to pneumoperitoneum in tuberculous patients with functionally normal lungs were significant at the 1 and 2 per cent level, and in many instances the mean difference was seven and eight times larger than the standard error. The opposite was true of changes due to pneumoperitoneum in tuberculous patients and in those with moderately impaired function. Here the 5 per cent level was never reached, and the chances were good that the mean difference was due to accidental sampling. The magnitude and direction of changes during pregnancy and during pneumoperitoneum in subjects with functionally normal lungs were almost identical. The functional residual capacity was reduced by 295 cc. in pregnant women at term and by 275 cc. after fully established pneumoperitoneum in patients with functionally normal lungs. The reduction of the lung volume on forced expiration was striking after pneumoperitoneum had been established. Assuming a dead space of 140 cc., the lungs in this position often contained less than 500 cc. of gas (table 2). The markedly atelectatic appearance of the lungs on chest roentgenograms exposed during maximal expiration (figure 6) is much like that seen during the last month of pregnancy (1). The diminution of lung volume in the pulmonary resting position in patients with significant functional impairment was only half as large as that of the previous two groups. The total lung capacity was strikingly stable in the face of full-term pregnancy or large pneumoperitoneum.

Inspection of individual patient responses (table 2) suggests that lung volume alterations due to pneumoperitoneum in patients with functionally normal lungs were slightly more uniform, both in direction and in magnitude, than the changes due to pregnancy in normal subjects. This may have been due in part to the more stable environment of the hospitalized patients. The volume changes due to pneumoperitoneum in patients with impaired pulmonary function were so slight that statistically significant trends could not be discerned. Comparison of roentgenograms, fluoroscopic findings, and physiologic studies permitted certain conclusions concerning the influence of disease on the physiologic outcome of pneumoperitoneum treatment. More extensive analyses will be required before generalizations can be made concerning the indications and probable effectiveness of artificial pneumoperitoneum treatment in the presence of advanced pulmonary tuberculosis.

In the present study, the problem of the effect of pregnancy and parturition in pulmonary tuberculosis has been approached solely from the standpoint of pulmonary physiology, and numerous factors (physiologic, immunologic, and sociologic) which might affect the course of the disease in individual patients have been ignored. The observations may be interpreted, however, as supporting the prevailing clinical impression that the reduction of the volume of the lungs in pulmonary resting position during the later months of pregnancy exerts a favor-

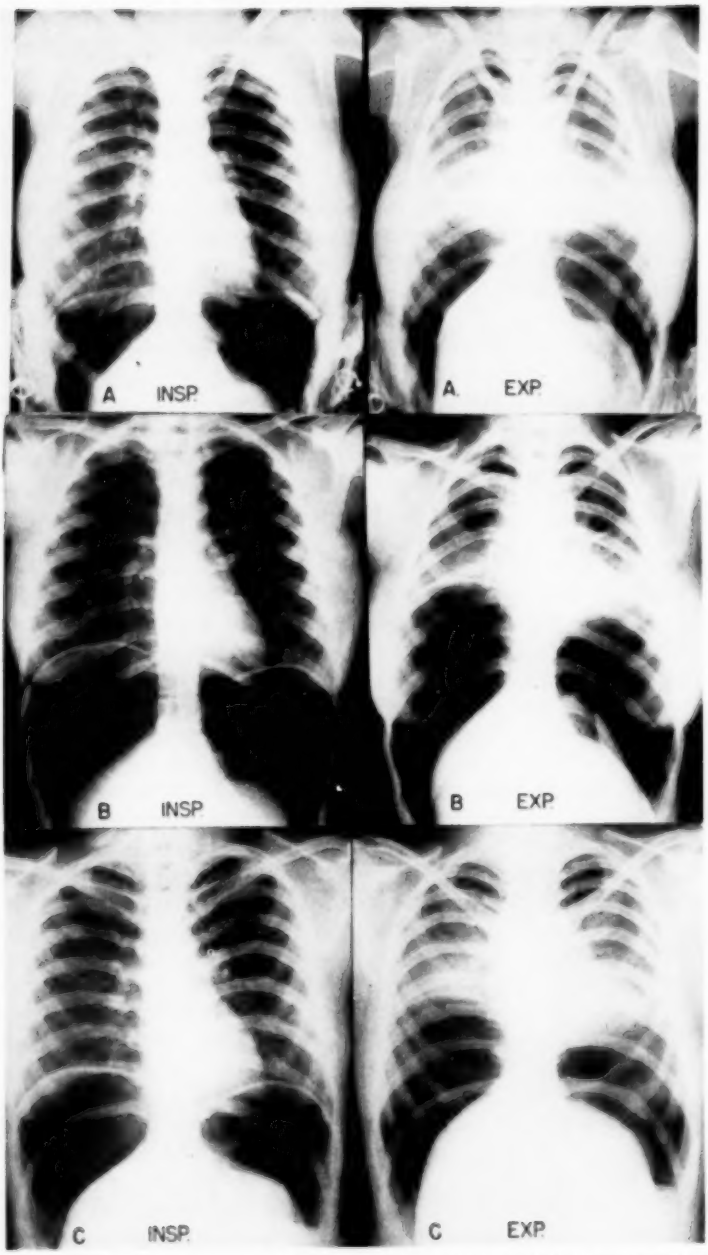

FIG. 6. Chest roentgenograms of Patients 8, 3, and 1, all with "functionally normal lungs" (table 2), during pneumoperitoneum treatment. The films on the left were exposed during full inspiration. At the end of forced expiration (right) the lung fields appeared almost atelectatic because the lungs contained only 320 cc. (*A*), 400 cc. (*B*), and 555 cc. (*C*) of gas if it is assumed that the dead space was 140 cc.

able influence on pulmonary tuberculosis, and that the loss of this effect at the time of delivery may be responsible for relapse or progression of disease. The evidence may be summarized as follows:

Pneumoperitoneum is widely believed to promote the healing of pulmonary tuberculous lesions. The present studies show that, like other therapeutic collapse measures, pneumoperitoneum diminishes the resting volume of the lung. This common factor is generally thought to account for the beneficial effects of these procedures. Pregnancy has been found to cause changes in the pulmonary volumes which are almost identical with those caused by pneumoperitoneum. Arguing by analogy, it seems likely that these alterations would exert a favorable effect on pulmonary tuberculosis. The respiratory apparatus reverts to its usual state of functioning soon after delivery. The "collapse" effect caused by the pregnant uterus is lost.

The favorable conditions which were present during pregnancy could be duplicated by the induction of postpartum pneumoperitoneum in the patients studied.

It should be pointed out that these patients in whom observations were made during pregnancy, after delivery, and after postpartum pneumoperitoneum had minimal tuberculosis and normal pulmonary function. Pneumoperitoneum caused less consistent alterations of pulmonary volumes in patients with more advanced disease. This was especially true when the process had been accompanied by extensive fibrotic changes, pleural involvement, or diaphragmatic fixation.

SUMMARY

Pulmonary ventilation and volumes, before and after induction of pneumoperitoneum, were studied in 23 young women who were hospitalized for treatment of active pulmonary tuberculosis.

Nine patients with minimal to moderately advanced tuberculosis, who were found to have functionally normal lungs, responded to pneumoperitoneum by an increase of inspiratory capacity and a decrease of expiratory reserve and residual volumes. The resulting decrease of the functional residual capacity (or collapse) was 17 per cent. The total lung capacity and vital capacity remained unchanged. These changes in lung volumes were similar to those seen in pregnancy. In contrast to pregnancy, which is accompanied by an increase in tidal volume, minute ventilation, and oxygen consumption, pneumoperitoneum resulted in a slight decrease of tidal volume and minute ventilation and no change in oxygen consumption. A significant increase in maximum breathing capacity was seen after pneumoperitoneum.

A second group of 9 patients with moderately to far advanced tuberculosis had pulmonary functional impairment of the restrictive type. They showed similar but smaller and more variable changes in expiratory reserve, residual volume, and functional residual capacity after induction of pneumoperitoneum. Collapse of the lung in the resting position was achieved in most cases, but the effect was smaller than that obtained in patients with functionally normal lungs. Failure of pneumoperitoneum to produce the expected collapse in some cases in this group could be related to fixation of the lungs, thoracic cage, or diaphragm. Tidal volume and resting ventilation decreased slightly while a decrease in maximum breathing capacity was not significant.

Five patients with minimal tuberculosis and normal lung function were studied serially during pregnancy and after induction of pneumoperitoneum post partum. During pregnancy pulmonary function followed the pattern seen in healthy women. The pulmonary collapse which accompanied pregnancy at term could be maintained quantitatively by induction of pneumoperitoneum post partum.

SUMARIO

La Función Pulmonar en el Embarazo. II. Comparación de los Efectos del Neumoperitoneo y del Embarazo en las Jóvenes que Tienen Pulmones de Función Normal y de las Observaciones Seriadas durante el Embarazo y el Neumoperitoneo Postpártum

En 23 mujeres jóvenes hospitalizadas para tratamiento de una tuberculosis pulmonar activa, se estudiaron la ventilación pulmonar y los volúmenes de la misma, antes y después de la inducción del neumoperitoneo.

Nueve enfermas con tuberculosis que variaba de mínima a moderadamente avanzada y que, según se observó, tenían pulmones de funcionamiento normal, respondieron al neumoperitoneo con aumento de la capacidad inspiratoria y disminución de los volúmenes de la reserva expiratoria y del aire residual. La disminución resultante de la capacidad residual funcional (o colapso) representó 17 por ciento. La capacidad pulmonar total y la capacidad vital permanecieron inalteradas. Esos cambios de los volúmenes pulmonares fueron semejantes a los observados durante la gestación. En contraposición a ésta, que va acompañada de aumento del volumen respiratorio, de la ventilación por minuto y del consumo de oxígeno, el neumoperitoneo dió por resultado leve disminución del volumen respiratorio y de la ventilación por minuto, pero sin cambio del consumo de oxígeno. Después del neumoperitoneo, notóse un aumento significativo de la capacidad respiratoria máxima.

Otro grupo de 9 enfermas con tuberculosis que variaba de moderadamente a muy avanzada tenían insuficiencia pulmonar de la forma restrictiva, revelando, después de la inducción del neumoperitoneo, alteraciones semejantes, pero menores y más variables, en la reserva expiratoria, el volumen residual y la capacidad residual funcional. En la mayor parte de los casos, obtúvose colapso del pulmón en la posición en descanso, pero el efecto fué menor que el obtenido en enfermas dotadas de pulmones que funcionaban normalmente. La incapacidad del neumoperitoneo para producir el colapso esperado en algunos casos de este grupo pudo ser relacionada con la fijación de los pulmones, de la caja torácica o del diafragma. El volumen respiratorio y la ventilación en reposo disminuyeron ligeramente, mientras que no revistió importancia la disminución de la capacidad respiratoria máxima.

Cinco enfermas que tenían tuberculosis mínima y función pulmonar normal fueron objeto de estudios seriados durante el embarazo y después de la inducción puerperal del neumoperitoneo. Durante la gestación, la función pulmonar se conformó al patrón observado en mujeres sanas. El colapso pulmonar que acompañó al embarazo a término pudo ser mantenido cuantitativamente con la inducción postpártum del neumoperitoneo.

RESUME

Fonction pulmonaire dans la grossesse. II. Comparaison des effets du pneumopéritoine et de la grossesse chez les jeunes femmes dont la fonction pulmonaire est normale et observations en séries au cours de la grossesse et du pneumopéritoine post-partum

La ventilation et les capacités pulmonaires, avant et après la création du pneumopéritoine, ont été étudiés chez 23 jeunes femmes hospitalisées pour traitement d'une tuberculose pulmonaire en activité.

Neuf malades présentant une tuberculose d'un degré compris entre minime et moyennement avancé et dont la fonction pulmonaire était considérée normale, ont répondu au pneumopéritoine par un accroissement de la capacité inspiratoire et une diminution de la

réserve expiratoire et des capacités résiduelles. La diminution de la capacité fonctionnelle résiduelle (ou collapsus) qui en est résultée était de 17%. La capacité pulmonaire totale et la capacité vitals sont demeurées inchangées. Ces modifications des capacités pulmonaires étaient semblables à celles observées dans la grossesse. Par contraste avec la grossesse, qui s'accompagne d'une augmentation du volume de l'air courant, de la ventilation par minute et de la consommation d'oxygène, le pneumopéritoine a produit une légère réduction du volume de l'air courant et de la ventilation par minute, sans aucun changement dans la consommation d'oxygène. Un accroissement significatif de la capacité respiratoire maxima a été observé après le pneumopéritoine.

Un deuxième groupe de 9 malades, dont la tuberculose était d'un degré compris entre "modéré" et "trés avancé," présentait un trouble fonctionnel pulmonaire d'un caractère restrictif. Après l'instauration du pneumopéritoine, ces malades montraient des modifications analogues, mais plus réduites et plus variables, de la réserve expiratoire, du volume résiduel et de la capacité résiduelle fonctionnelle. Le collapsus du poumon en position de repos était réalisé dans la plupart des cas, mais l'effet obtenu était plus faible que celui observé chez les malades dont la fonction pulmonaire était normale. L'échec du pneumopéritoine à produire le collapsus prévu, dans certains cas de ce groupe, pouvait être en relation avec la fixation du poumon, de la cage thoracique ou du diaphragme. Le volume de l'air courant et la ventilation au repos ont diminué légèrement tandis que la diminution de la capacité respiratoire maxima était insignifiante. Il a été fait une étude en série de 5 malades atteintes de tuberculose *a minima* dont la fonction pulmonaire était normale, d'abord au cours de la grossesse et après l'instauration d'un pneumopéritoine post-partum. Au cours de la grossesse, la fonction pulmonaire était semblable à celle observée chez les femmes saines. Le collapsus pulmonaire qui accompagnait la grossesse à terme, pouvait être maintenu quantitativement par la création du pneumopéritoine post-partum.

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PULMONARY FUNCTION IN PREGNANCY[1, 2, 3]

III. Serial Observations in Patients with Pulmonary Insufficiency

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INTRODUCTION

The hazard incurred by burdening a damaged respiratory system with the additional demands of pregnancy has never been adequately defined. In recent years surgical excision or collapse measures together with chemotherapy preserved the lives of many young women with advanced pulmonary tuberculosis. Arrest of the disease has been achieved in many cases at the expense of considerable loss of pulmonary function. The ability of these patients to bear children safely is often questioned.

In normal women the ventilatory capacity does not appear to be diminished by pregnancy (1). The oxygen uptake is increased by the necessity of carrying the weight of the fetus and because the respiratory needs of the fetus must be met by the mother. The larger oxygen consumption requires increased pulmonary ventilation and this adjustment must be made in the presence of a somewhat altered abdomino-thoracic pressure relationship. How this is accomplished by the normal mother has been described in the first paper of this series (1). The present study was undertaken to ascertain whether in women with pulmonary insufficiency the ventilatory capacity remains unaffected during pregnancy and whether the functionally impaired respiratory apparatus makes the adjustments to the greater ventilatory work with more or less economy than the normal one.

Numerous clinical reports of successful pregnancy in women with limited breathing capacity suggest that an impaired respiratory apparatus is more competent in dealing with the additional respiratory demands than the damaged heart is in meeting the increased circulatory demands. Although disease of the lungs alone is probably the most frequent cause of a limited breathing reserve during pregnancy, attention is not usually focused on pulmonary function unless the patient has had treatment by collapse or resection. Forlanini, in

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1906, first reported a successful pregnancy during the course of artificial pneumothorax treatment (2). During the years of enthusiasm for pneumothorax, many cases were reported in which treatment was continued during pregnancy (3). Clinical evidence of pulmonary insufficiency was apparently so rare as not to deserve mention. Even more surprising is the low incidence of dyspnea in pregnant patients with bilateral pneumothorax (4).

There seems to be little danger of respiratory difficulty during pregnancy in patients who have had thoracoplasty. Seeley and associates, in 1940, collected from the literature reports of thirty-one women who had thirty-four pregnancies after operation (5). Dyspnea was mentioned only three times in the reports and was present in four of their own ten cases. When respiratory difficulties did occur, the symptoms progressed and were usually most severe at term and during delivery. This is in contrast to normal women in whom there appears to be no close relationship between the severity of dyspnea and the stage of pregnancy (1). Respiratory symptoms were more prone to occur in patients who had nonselective total lateral thoracoplasties. In these patients labor was often accompanied by an alarmingly high pulse rate, and operative intervention was required to hasten delivery (6). Thoracoplasty during pregnancy has been performed many times and is well tolerated from the respiratory standpoint (7, 8).

Until recently, extensive pulmonary resections in women of child-bearing age were rare. Pregnancy following pneumonectomy for pulmonary tuberculosis has apparently not been reported although, at present, this must be a not infrequent occurrence. The present writers have had the opportunity to follow eight women who had uneventful pregnancies and normal deliveries after pneumonectomy (9). In the single reported case of pregnancy after pneumonectomy, the operation was performed for adenocarcinoma (10). This 37-year-old woman was studied at monthly intervals during pregnancy by spirometry, maximum breathing capacity, venous pressure, and saline infusion tests. Increasing dyspnea on exertion was observed, but this case cannot be considered typical. The remaining lung must have been severely damaged because the vital capacity of 1,050 cc. and the maximum breathing capacity between 17 and 22 liters per minute were only one-half as large as similar measurements in other patients after pneumonectomy (11).

Previous studies of pulmonary function of normal pregnant women were not planned with a view to establishing the necessary pulmonary reserve for safe pregnancy. Anthony and Hansen believed that pregnancy decreased the respiratory reserve because of an increased minute ventilation and a decreased vital capacity (12). They thought that pregnancy might well produce respiratory decompensation and hypoxia in patients with extensive pulmonary disease, kyphoscoliotic emphysema, or pleuritis. The studies by the present writers of normal subjects lead to the belief that pregnancy should be well tolerated, even in the face of an exceedingly low breathing reserve, provided that patients with pulmonary insufficiency show a respiratory response to pregnancy similar to that of normal women (1).

PLAN OF INVESTIGATION

Selection of Patients

Seven patients with diminished pulmonary function due to tuberculosis and operative collapse or resection were studied on 117 occasions. Observations were made before, fourteen days and six months after surgery, later at yearly intervals, and again early and late during the course of nine pregnancies.

The patients were selected from a group of 142 women in the child-bearing age (seventeen to forty-four years) who were operated upon for the treatment of pulmonary tuberculosis at the Sanatorium Division of the Boston City Hospital between November, 1947, and November, 1952. Of this group, 72 per cent had a measurable degree of ventilatory insufficiency before operation and 89 per cent had diminished function six months after operation. The 7 patients described in this study were the only women of this group who had demonstrably impaired function and who became pregnant during the five-year observation period.

Method

The techniques employed for the evaluation of pulmonary function have been described in the first paper of this series (1). The functional residual capacity and the intrapulmonary mixing index were not routinely determined before and after surgery unless there was evidence of obstructive pulmonary insufficiency. More extensive studies were initiated when pregnancy was recognized. Before and after pneumonectomy, all tests customarily used in this laboratory were employed and the studies were repeated at monthly, rather than yearly, intervals (11). In addition to the previously mentioned studies, bronchospirometry was performed in all of the 7 patients. The technique used in performing split-function studies has been described in a previous report (13).

RESULTS

The patients were divided into two groups according to the degree of ventilatory insufficiency found long after operation and before pregnancy. The 4 patients in the first group had moderate insufficiency, with maximum breathing capacities which were 75 to 90 per cent of the predicted normal and/or vital capacities which were 60 to 75 per cent of the predicted normal (table 1). The 3 patients in the second group and an additional patient from the first group, during a second pregnancy, had severe restrictive insufficiency with maximum breathing capacities which were 50 to 75 per cent of predicted and/or vital capacities which were 40 to 60 per cent of predicted (table 2).

The results of studies of each patient, on selected occasions, are shown in tables 1 and 2. Mean values for the two groups before and at the end of pregnancy, and the observed changes (expressed in per cent) are summarized in table 3. Observations of 19 normal women, taken from the first paper of this series (1), are shown for comparison.

TABLE 1

BREATHING CAPACITY AND LUNG VOLUMES OF PREGNANT WOMEN WITH MODERATE PULMONARY INSUFFICIENCY

PATIENT	AGE	DATE	CONDITION	MBC, PER CENT OF PREDICTED	VC, PER CENT OF PREDICTED	AIR VELOCITY INDEX	WALKING INDEX PER CENT	INSPIRATORY CAPACITY, CC.	EXPIRATORY RESERVE, CC.	VITAL CAPACITY, CC.	FUNCTIONAL RESIDUAL CAPACITY, CC.	TOTAL LUNG CAPACITY, CC.	R.V. T.L.C. × 100, PER CENT	MIXING INDEX, PER CENT N_2
N. D.	19	December 1947	Far advanced pulmonary tuberculosis, left unexpandable pneumothorax	79	71	1.11	21	—	—	2,320	—	—	—	—
	20	July 1948	6 months after left 7-rib thoracoplasty	77	66	1.17	22	—	—	2,170	—	—	—	—
	20	January 1950	18 months after discharge, 2 months pregnant	81	67	1.21	17	1,750	440	2,190	1,250	3,000	27	1.16
	21	August 1950	9 months pregnant	80	70	1.14	23	1,930	390	2,320	1,080	3,010	23	1.02
	23	July 1952	22 months post partum	81	67	1.21	17	1,760	460	2,220	1,320	3,080	28	1.27
F. Mc.	22	February 1950	Moderately advanced pulmonary tuberculosis	96	90	1.06	14	—	—	3,250	—	—	—	—
	23	August 1950	6 months after extraperiosteal plombage	83	69	1.20	23	—	—	2,310	—	—	—	—
	24	August 1951	1 year after discharge	83	69	1.20	21	1,980	350	2,330	1,090	3,070	24	0.87
	24	January 1952	2 months pregnant	78	75	1.04	26	2,180	320	2,500	1,110	3,290	24	0.80
	25	August 1952	9 months pregnant	76	77	0.99	29	2,310	250	2,560	970	3,280	22	0.67
A. D.	24	October 1948	2 months pregnant, left fibrothorax due to therapeutic pneumothorax	97	96	1.01	15	2,350	500	2,850	1,200	3,550	20	—
	24	February 1949	6 months pregnant	101	95	1.06	16	2,300	450	2,750	1,090	3,390	19	—
	25	May 1949	9 months pregnant	105	87	1.20	21	2,100	450	2,550	940	3,040	16	0.81
	25	June 1949	2 weeks post partum	102	87	1.16	17	2,130	480	2,610	1,230	3,360	22	1.32

B. S.	21	May 1949	Far advanced pulmonary tuberculosis, 6 weeks after pneumoperitoneum	104	84	1.24	16	2,280	420	2,700	—	—	—	—
	22	December 1949	6 months after right 7-rib thoracoplasty	96	59	1.63	20	—	—	1,900	—	—	—	—
	24	July 1951	2 years after thoracoplasty	105	64	1.64	22	1,760	310	2,070	1,040	2,800	26	0.92
	25	July 1952	9 months pregnant	103	64	1.61	27	1,800	250	2,050	860	2,660	23	0.62
Mean, 0 to 2 months pregnant				90	76	1.23	20	2,010	393	2,403	1,150	3,160	24	0.96
Mean, 9 months pregnant				91	75	1.24	25	2,035	335	2,370	963	2,998	21	0.77

MBC = maximum breathing capacity.
VC = vital capacity.

TABLE 2

BREATHING CAPACITY AND LUNG VOLUMES OF PREGNANT WOMEN WITH SEVERE PULMONARY INSUFFICIENCY

PATIENT	AGE	DATE	CONDITION	MBC, PER CENT OF PREDICTED	VC, PER CENT OF PREDICTED	AIR VELOCITY INDEX	WALKING INDEX, PER CENT	INSPIRATORY CAPACITY, CC.	EXPIRATORY RESERVE, CC.	VITAL CAPACITY, CC.	FUNCTIONAL RESIDUAL CAPACITY, CC.	TOTAL LUNG CAPACITY, CC.	R.V./T.L.C. × 100, PER CENT	MIXING INDEX, PER CENT N_2
A. D.	25	February 1950	7 months after thoracoplasty, right	81	61	1.33	20	1,510	300	1,810	810	2,350	23	1.67
	26	November 1950	7 months after pneumonectomy, left	49	40	1.23	34	1,180	115	1,295	575	1,755	26	0.69
	27	December 1951	12 months after discharge	50	41	1.20	37	1,290	150	1,140	510	1,800	20	0.80
	28	July 1952	7 months pregnant	49	42	1.17	48	1,450	50	1,500	380	1,830	18	0.61
I. C.	24	November 1948	Giant cavity, right apex, 50 per cent pneumothorax, left	87	57	1.53	22	—	—	1,820	—	—	—	—
	25	July 1949	6 months after right thoracoplasty	73	41	1.78	27	—	—	1,300	—	—	—	—
	26	June 1950	2 months pregnant	74	42	1.76	28	1,170	160	1,330	610	1,780	25	0.86
	27	June 1951	9 months pregnant	72	41	1.76	39	1,210	90	1,300	400	1,610	19	0.66
	27	June 1951	4 months pregnant	73	43	1.70	29	1,170	210	1,380	620	1,790	23	0.95
	28	July 1952	9 months pregnant	72	41	1.76	40	1,200	100	1,300	390	1,590	18	0.75
O. F.	26	June 1949	Destroyed right lung	99	66	1.50	23	1,370	630	2,000	1,755	3,125	36	2.82
	26	September 1949	3 months after right pneumonectomy and thoracoplasty	70	51	1.37	33	1,000	310	1,310	980	1,980	34	1.26
	28	November 1951	2 years later, 8 months pregnant	63	49	1.29	38	1,100	290	1,390	745	1,845	25	0.39

A. B.	29	July 1950	Left apical disease, 7 years after right thoracoplasty	65	60	1.08	24	1,200	400	1,800	1,210	2,610	31	2.11
	29	December 1950	6 months after left extrapleural pneumothorax	62	50	1.24	25	1,110	400	1,510	830	1,940	22	1.20
	30	July 1951	6 months after discharge, 1 month pregnant	63	49	1.23	26	1,140	390	1,530	820	1,960	22	0.90
	31	April 1952	9 months pregnant	60	53	1.13	31	1,300	290	1,590	730	2,030	22	0.96
	31	July 1952	3 months post partum	61	49	1.24	24	1,100	370	1,470	780	1,880	22	1.05
Mean, 0 to 2 months pregnant				66	46	1.45	31	1,154	244	1,398	708	1,862	25	0.95
Mean, 7 to 9 months pregnant				63	45	1.42	39	1,252	164	1,416	529	1,781	20	0.67

MBC = maximum breathing capacity.
VC = vital capacity.

TABLE 3

COMPARISON OF CHANGES IN PULMONARY FUNCTION DUE TO PREGNANCY IN NORMAL SUBJECTS AND IN PATIENTS WITH MODERATE AND SEVERE PULMONARY INSUFFICIENCYV

DETERMINATION	PREGNANCY, NORMAL VOLUNTEERS			PREGNANCY, MODERATE PULMONARY INSUFFICIENCY			PREGNANCY, SEVERE PULMONARY INSUFFICIENCY		
	Control	Term	Per Cent Change	Control	Term	Per Cent Change	Control	Term	Per Cent Change
Number of patients	19			4			5		
Age, years	22			24			28		
Body surface, m^2	1.64	1.76	+7	1.68	1.80	+7	1.59	1.68	+6
Respiratory rate	15	16	+10	14	15	+7	21	24	+14
Tidal volume, cc	487	678	+39	505	635	+26	320	350	+9
Resting ventilation, L.	7.27	10.34	+42	7.07	0.52	+35	6.72	8.40	+25
Resting oxygen uptake, cc.	201	266	+32	226	286	+27	204	263	+29
Ventilatory equivalent, L/100 cc	3.0	3.3	+10	2.9	2.8	−4	2.8	2.7	−4
Breathing reserve, per cent.	92	89	−3	92	89	−3	88	85	−3
Walking ventilation, L/min.	14.7	19.1	+30	18.1	22.8	+26	18.3	21.8	+19
Walking index, per cent	15	21	+40	20	25	+25	31	39	+20
Maximum breathing capacity, L/min.	102	96	−6	90	91	+1	59	56	−5
Upright vital capacity, cc.	3,200	3,140	−2	2,430	2,400	−1	1,420	1,390	−2
Per cent of vital capacity, first second	84	82	—	83	83	—	84	86	—
Per cent of vital capacity, first 2 seconds	94	94	—	92	93	—	95	96	—
Per cent of vital capacity, first 3 seconds	98	97	—	98	99	—	100	100	—
Air velocity index	1.04	0.94	−10	1.23	1.24	+1	1.45	1.42	−2
Inspiratory capacity, cc.	2,625	2,745	+5	2,010	2,035	+1	1,154	1,252	+8
Expiratory reserve, cc.	655	555	−15	393	335	−15	244	164	−33
Supine vital capacity, cc.	3,280	3,300	+1	2,403	2,370	−1	1,398	1,416	+1

TABLE 3—*Continued*

DETERMINATION	PREGNANCY, NORMAL VOLUNTEERS			PREGNANCY, MODERATE PULMONARY INSUFFICIENCY			PREGNANCY, SEVERE PULMONARY INSUFFICIENCY		
	Control	Term	Per Cent Change	Control	Term	Per Cent Change	Control	Term	Per Cent Change
Residual volume, cc	965	770	−20	757	628	−17	464	365	−21
Functional residual capacity, cc	1,620	1,325	−18	1,150	963	−16	708	529	−25
Total lung capacity, cc	4,245	4,070	−4	3,160	2,998	−5	1,862	1,781	−4
R.V./T.L.C. × 100, per cent	23	19	−17	24	21	−12	25	20	−20
Intrapulmonary mixing index, per cent N_2	0.56	0.46	—	0.96	0.77	—	0.95	0.67	—

The Effect of Pregnancy in Patients with Moderate Pulmonary Insufficiency

One patient in this group (F. Mc.) had moderately advanced pulmonary tuberculosis, and the disease was far advanced in the other 3 patients. Selective seven-rib thoracoplasties contributed to the pulmonary insufficiency in 2 (N. D., B. S.) and a six-rib subcostal extraperiosteal Lucite sphere plombage in one patient (F. Mc.). The fourth patient had a poorly re-expanded left lung five years after a therapeutic pneumothorax had been abandoned (A. D.). In spite of maximum breathing and vital capacities of over 90 per cent of predicted, this lung contributed only 30 per cent to the total oxygen uptake (table 4).

Studies before pregnancy: Studies of this group before pregnancy revealed a slightly reduced maximum breathing capacity, moderate hyperventilation during exercise, and a low total lung capacity with symmetrical reduction of all of its subdivisions (table 3).

The 4 patients were comparable in age and stature to the normal subjects studied during pregnancy. At rest the respiratory rate was not elevated, oxygen uptake was normal, and there was no hyperventilation. The breathing reserve of 92 per cent was the same as in normal women (table 3). The ventilatory volume during exercise was 23 per cent greater than that of normal subjects. This and the slightly reduced maximum breathing capacity contributed to an elevated walking dyspnea index. The most constant abnormality was diminution of the lung volume. The total lung capacity was 78 per cent of predicted, and the subdivisions, inspiratory capacity, expiratory reserve and residual volume, shared about equally in this reduction. There was no evidence of emphysema or obstruction: the air velocity index, timed capacities, ratio of residual volume to total lung capacity, and the intrapulmonary mixing index were normal. In all of the patients of this group, functional impairment was due to unilaterally reduced maximal stroke volume (table 4).

Studies during pregnancy: Studies were made during the ninth month of

pregnancy. The observed changes were similar, both in magnitude and in direction, to those seen in normal subjects (table 3). A 35 per cent increase of resting ventilation was achieved by increasing the volume of tidal excursions. Unlike

TABLE 4

SPLIT FUNCTION STUDIES OF 7 PATIENTS WITH PULMONARY INSUFFICIENCY BEFORE SURGERY AND BEFORE PREGNANCY

PATIENT	DATE	CONDITION	OXYGEN UPTAKE PER CENT OF TOTAL		VENTILATION PER CENT OF TOTAL		VITAL CAPACITY PER CENT OF TOTAL	
			Right Lung	Left Lung	Right Lung	Left Lung	Right Lung	Left Lung
N. D.	July 1948	After 7-rib thoracoplasty, left	*74*	*26*	*64*	*36*	*72*	*28*
F. Mc.	February 1950	Moderately advanced disease, right lung	45	55	50	50	46	54
	June 1950	After 6-rib subcostal plombage right	*42*	*58*	*51*	*49*	*44*	*56*
A. D.	October 1948	Left pneumothorax, unexpandable, 10 years	*70*	*30*	*72*	*28*	*73*	*27*
	February 1950	Right 5-rib thoracoplasty, progressive disease, left	75	25	62	38	76	24
		(after left pneumonectomy)	*100*	*0*	*100*	*0*	*100*	*0*
B. S.	May 1949	Far advanced disease, right lung	40	60	46	54	41	59
	December 1949	After 7-rib thoracoplasty, right	*29*	*71*	*36*	*64*	*31*	*69*
I. C	November 1948	Far advanced disease right; 50 per cent pneumothorax, left	53	47	53	47	56	44
	August 1949	After 6-rib thoracoplasty, right	*41*	*59*	*42*	*58*	*45*	*55*
O. F.	February 1949	Far advanced disease, right lung	20	80	39	61	28	72
		(after right pneumonectomy)	*0*	*100*	*0*	*100*	*0*	*100*
A. B.	July 1950	Far advanced disease, left; 7-rib thoracoplasty, right	19	81	26	74	16	84
	December 1950	After extrapleural pneumothorax, left	*21*	*79*	*26*	*74*	*25*	*75*

Figures in italics indicate functional division at the time of pregnancies.

normal subjects, these patients increased minute ventilation only enough to supply the augmented oxygen demand, and the ventilatory equivalent was not larger than before pregnancy. Also ventilation during exercise did not increase quite as much as in normal subjects. Maximum breathing capacity and vital

capacity remained unaffected, and the subdivisions of the total lung capacity showed the changes characteristic of increased intra-abdominal pressure: reduction of expiratory reserve and residual volumes, and slight increase of inspiratory capacity. In the pulmonary resting position, the volume of the lungs was 187 cc. less than during the control period, and the residual volume occupied only 21 per cent of the total lung capacity. The somewhat lower intrapulmonary mixing index at term was attributed to the increased ventilation (1).

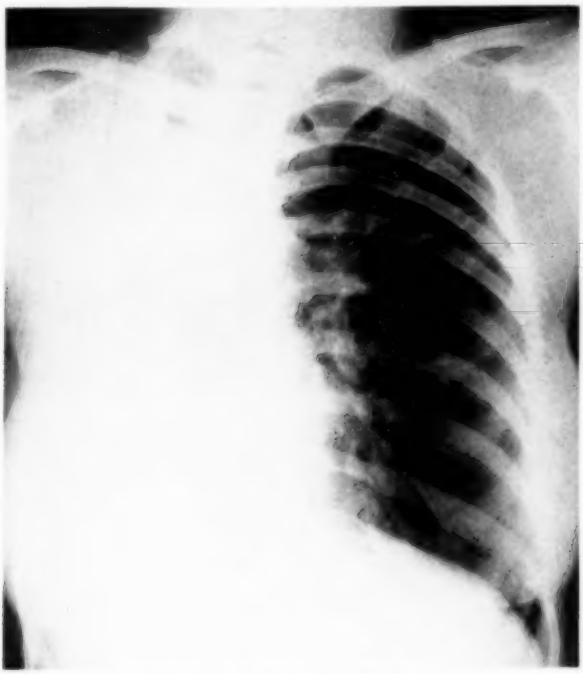

FIG. 1. Chest roentgenogram of patient O. V., a 26-year-old woman with severe pulmonary insufficiency. The film was obtained six months after pneumonectomy and space-filling thoracoplasty and one year before pregnancy.

The Effect of Pregnancy in Patients with Severe Pulmonary Insufficiency

Severe restrictive insufficiency is rarely seen if disease is limited to one lung. Only one patient in this group (O. V.) had surgery on only one side, but the remaining lung had at one time been extensively diseased (figures 1 and 2). The other 3 patients became pregnant after bilateral surgery. Patient A. D., who had been studied during one previous pregnancy (table 1), became pregnant again three years later. In the meantime, a five-rib thoracoplasty had been performed on the right, and the unexpanded diseased lung on the left had been removed. The very limited pulmonary reserve of the third patient (I. C.) was due to an extensive therapeutic pneumothorax on the left side and a six-rib

thoracoplasty on the right (figures 3 and 4). The last patient (A. B.) had a poorly selective seven-rib thoracoplasty on the right seven years before pregnancy and an extrapleural pneumothorax on the left six years later (table 2).

Studies before pregnancy: Comparison of data obtained before pregnancy in this group with those of the 19 normal subjects revealed a severe functional impairment (table 3). The respiratory rate was elevated at rest. Ventilation remained the same as in normal subjects because the tidal excursions were smaller. Oxygen uptake was normal in relation to ventilatory volume and estab-

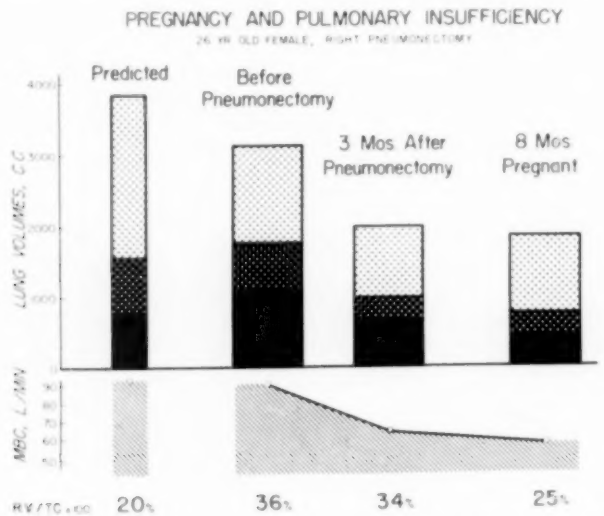

FIG. 2. Maximum breathing capacity and subdivisions of the total lung capacity of patient O. F. before and after pneumonectomy and during the eighth month of pregnancy. "Normal" predicted values are indicated by the column on the left. In this illustration and in figure 4 the subdivisions of the total lung capacity are identified as follows: black—*residual volume*; stippled black—*expiratory reserve*; stippled white—*inspiratory capacity*. The dark areas taken together represent *functional residual capacity*, and the stippled areas together, *vital capacity*.

lished basal metabolic rate. The 88 per cent breathing reserve was less than the lower limit of normal. Hyperventilation during exercise was of the same order as in the previous group, and the walking dyspnea index of 31 per cent was approximately twice normal.

The mean maximum breathing capacity was 59 liters per minute, or 66 per cent of the predicted value. The vital capacity in the upright position was 1,120 cc., or 46 per cent of the predicted value. The air velocity index, slightly elevated in the group with moderate insufficiency, was now greatly elevated. Spirometry and residual volume determinations showed all subdivisions of the total lung capacity equally reduced. Poor function in this group was due to a severe restriction of the maximal stroke volume, which was bilateral in 3 patients and uni-

lateral in one (table 1). Again there was no evidence of obstruction or emphysema: the timed capacities, ratios of residual volume to total lung capacity, and intrapulmonary mixing indices remained within normal limits.

Studies during pregnancy: Observations were made in the eighth month of pregnancy in two instances (Patients A. D., O. F.) and during the ninth month in the remaining 3 patients. The response of the markedly impaired respiratory

FIG. 3. Chest roentgenogram of patient L. C., a 24-year-old woman with severe pulmonary insufficiency. The film was taken four years after induction of therapeutic pneumothorax on the left and six months after a 6-rib thoracoplasty on the right. The patient had a normal delivery two years and, again, three years after this date.

apparatus to increased intra-abdominal pressure differed in many respects from that observed in normal subjects or in patients with less pulmonary dysfunction.

Resting ventilation was increased only 25 per cent. In patients with moderate pulmonary insufficiency, this volume was increased by 35 per cent; and in normal subjects, by 42 per cent. In striking contrast to the other two groups, the greater minute ventilation was largely due to an increase in respiratory rate. The tidal volume increased only 9 per cent. Only in the present group was the increase of oxygen demand larger than the increase of ventilation. The ventilatory equivalent was therefore reduced at term. The expected increase in exercise ventilation was also less marked than in the previous group. The walking dyspnea

index rose above the "dyspnea level" of 35 per cent because of the low maximum breathing capacity.

The remaining studies revealed responses similar to those observed in the previous group and in normal subjects. The maximum breathing capacity and upright vital capacities remained unchanged. The subdivisions of the total lung capacity showed much the same alterations as in normal women. The per cent changes were exaggerated because the initial volumes were smaller. The volume of the lungs in the pulmonary resting position was decreased by 180 cc., and the residual volume occupied 20 per cent of the total lung capacity. The intrapulmonary mixing index again showed a downward trend.

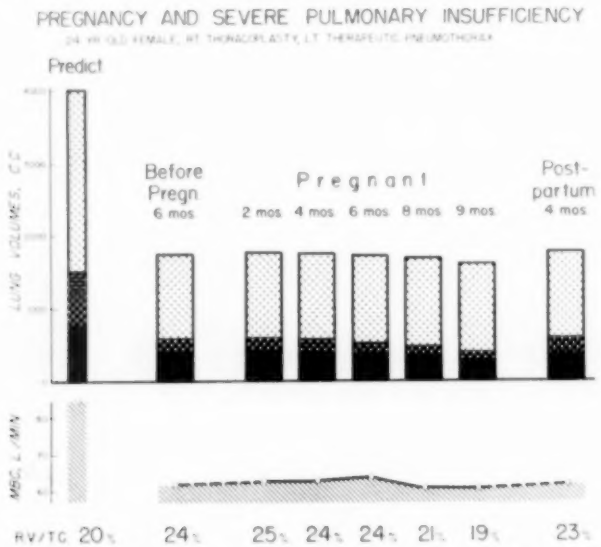

FIG. 4. Maximum breathing capacity and subdivisions of the total lung capacity before, at intervals during, and four months after the first pregnancy of patient I. C. "Normal" predicted values are indicated by the column on the left. The code is the same as in figure 2.

Dyspnea during pregnancy: The most unexpected finding in these patients was the absence of dyspnea, whereas 68 per cent of the normal subjects had complained of shortness of breath at some time during pregnancy (1).

The 4 patients with moderate pulmonary insufficiency had no limitation of activity before pregnancy. All were able to climb several flights of stairs without rest and, in their judgment, without undue shortness of breath. Exercise tolerance was not changed during pregnancy, and repeated questions concerning dyspnea produced no positive response.

The 4 patients with severe pulmonary insufficiency were definitely limited in their activity after completion of the surgical program. Patients A. D. and I. C. reported some shortness of breath while walking on the level. The other 2

patients could not climb an entire flight of stairs without resting. After their return home, exercise tolerance did not increase but they complained less of dyspnea as they learned to adapt their activity to their pulmonary reserve. This group reported no change in limitation of activity or dyspnea during pregnancy and all of the 4 patients continued their daily housework until term. All nine deliveries were conducted according to the preferences of the obstetrical staff of the local lying-in hospitals. No alarming signs referable to respiratory or circulatory systems were noted during labor.

DISCUSSION

The ventilatory capacity of these pregnant women with pulmonary insufficiency was reduced because of a diminution of maximal stroke volume. This type of "restrictive" ventilatory insufficiency is usually well tolerated. Both limitation of activity and shortness of breath may be expected to decrease in time. Although the maximal stroke volume (or vital capacity) is irreparably diminished, the maximal breathing capacity is not as severely impaired. This might be expected because these patients breathe more rapidly and regularly during stress and the respiratory passages offer relatively less resistance to the smaller maximal stroke volume (14). These observations are confirmed by an air velocity index which is larger than the normal 1.0 (table 3).

The conclusions from the present study of the effects of pregnancy on patients with restrictive pulmonary insufficiency do not necessarily apply to women who have diminished ventilatory capacity because of an obstructive ventilatory defect. The reaction to pregnancy of patients with loss of pulmonary elasticity or increased resistance to air flow might differ greatly from those with restrictive insufficiency. Even minor obstructive defects are usually poorly tolerated. Limitation of activity and dyspnea usually increase with time, and obstructive defects have disabling sequelae which are not prominent in restrictive insufficiency.

Comparison of the effect of pregnancy on the respiratory apparatus of normal subjects with the effect on patients with pulmonary insufficiency permits three generalizations:

1. Hyperventilation becomes less marked as pulmonary insufficiency increases. In normal subjects at term, resting ventilation was increased 42 per cent; in patients with moderate pulmonary insufficiency, 35 per cent; and in patients with severe pulmonary insufficiency, 25 per cent. In the most severely disabled patients, there was no hyperventilation because ventilation increased no more than oxygen uptake. The same trend was observed during the standard exercise: at term, ventilation was increased 30 per cent in normal subjects, 26 per cent in the moderately limited group, and only 19 per cent in the severely disabled group.

2. The greater ventilatory requirement is met by an increase of tidal excursions in normal women and in patients with moderately impaired function. When pulmonary insufficiency is more severe, ventilation is increased chiefly by an increase of the respiratory rate. Although this causes a disadvantageous alveolar ventilation/total ventilation ratio, it is a more economical mode of increasing ventilation for patients with greatly

diminished lung volume. In normal subjects, with vital capacities of 3 to 4 liters, a doubled tidal volume requires only a doubled muscular effort; in other words, the greater intrapleural pressure change produces a correspondingly greater volume change. If patients with vital capacities of 1,200 or 1,300 cc. (table 1) double their tidal volume, the elastic limits of thoracic cage and lungs are reached at the peak of inspiration and expiration. In this region, increases in intrapleural pressure no longer result in a correspondingly greater tidal exchange.

3. The response of maximal stroke volume and ventilatory capacity to pregnancy was the same in patients with all degrees of pulmonary insufficiency and in normal subjects: maximum breathing capacity and vital capacity remained essentially unchanged. The subdivisions of the total lung capacity showed the changes characteristic of breathing with more deflated lungs. The degree of "collapse" or reduction of functional residual capacity was the same as in normal subjects.

In the present study, patients with pulmonary insufficiency, like normal women, showed no diminution of ventilatory capacity during pregnancy. The increment of ventilatory work at rest or during standard exercise was no greater at term than in normal subjects. On the contrary, the increment tended to be less in the more severely handicapped, although the small number of patients may not justify this generalization. It is therefore possible to predict how well individual patients with restrictive ventilatory insufficiency will tolerate pregnancy. In any patient, dyspnea becomes severe if the breathing reserve at rest is reduced below 60 to 70 per cent (15) or if the walking dyspnea index is increased above 50 per cent (16). Ventilatory capacity is not changed by pregnancy. Ventilatory requirement at rest is never increased by more than 40 per cent, or by more than 30 per cent during moderate exercise. If the maximum breathing capacity has been determined, breathing reserve and dyspnea indices at term can be predicted.

These studies also suggest that the respiratory stimulus during pregnancy is not augmented as much in patients with impaired pulmonary function as it is in normal subjects. This may account for the absence of subjective dyspnea during pregnancy in patients with pulmonary insufficiency. In clinical terms, these results imply that patients with restrictive ventilatory insufficiency, who are not dyspneic at rest or during slightest exertion, should tolerate pregnancy without difficulty. This conclusion is supported by the report of many successful pregnancies in patients who have undergone extensive collapse or excision therapy.

SUMMARY

Seven patients with diminished pulmonary function due to tuberculosis and operative collapse or resection were observed during the course of nine pregnancies. Pulmonary function studies were performed before, immediately after, and long after surgery. The studies were repeated before, during, and after pregnancy.

Observations before pregnancy revealed the presence of moderate to severe "restrictive" ventilatory insufficiency with diminished maximum breathing ca-

pacity and symmetrical reduction of the subdivisions of total lung capacity. There was no evidence of an "obstructive" defect. Timed capacities, ratios of residual volume to total lung capacity, and intrapulmonary mixing indices were within normal limits.

The functional alterations at term were compared with those observed in 19 normal pregnant women.

Normal women showed a pronounced hyperventilation at rest and during exercise. This tendency was less marked in patients with moderate pulmonary insufficiency, and was absent in patients with greatly impaired function. The increased oxygen requirement was met by greater tidal excursions in all but the most severely disabled patients, who increased ventilation by raising the respiratory rate.

The response to pregnancy of ventilatory capacity and lung volumes was the same in normal subjects and in patients with moderate and severe pulmonary insufficiency. In all three groups the maximal breathing capacity and vital capacity were the same at term as in the nonpregnant state. The subdivisions of the total lung capacity of all patients showed the changes characteristic of increased intra-abdominal pressure: the expiratory reserve and residual volumes were diminished and the inspiratory capacity was slightly increased.

In contrast to the normal subjects, the patients with moderate pulmonary insufficiency did not complain of dyspnea at any time during pregnancy. Patients with severely impaired function were not more disabled during pregnancy than before.

On the basis of the studies presented, the probable breathing reserve and dyspnea index of pregnant women at term can be calculated if the ventilatory capacity is known. It was concluded that patients with restrictive ventilatory insufficiency who are not dyspneic at rest should tolerate pregnancy without great difficulty. These conclusions do not necessarily apply to patients in whom ventilatory insufficiency is due to increased airway resistance or loss of pulmonary elasticity.

SUMARIO

La Función Pulmonar en el Embarazo: III. Observaciones Seriadas en Enfermas con Insuficienciencia Pulmonar

Siete enfermas con hipofunción pulmonar debida a la tuberculosis y al colapso operatorio o la resección fueron observadas durante el transcurso de nueve embarazos. Ejecutáronse estudios de la función pulmonar antes, inmediatamente después y mucho tiempo después de la operación. Los estudios fueron repetidos antes y después del embarazo y durante el mismo.

Las observaciones realizadas antes de la gestación revelaron insuficiencia "restrictiva" de la ventilación que variaba de moderada a grave, con disminución de la capacidad respiratoria máxima y reducción simétrica de las subdivisiones de la capacidad total del pulmón. No había signos de defecto "obstructivo." Las capacidades por minuto, las proporciones del volumen residual a la capacidad pulmonar total y los índices de la mezcla intrapulmonar quedaban dentro de límites normales.

Las alteraciones funcionales a término fueron comparadas con las observadas en 19 gestantes normales. Las últimas mostraron hiperventilación pronunciada en reposo y du-

rante el ejercicio. Esa tendencia fué menos notable en las pacientes que tenían moderada insuficiencia pulmonar y no existía en las que tenían la función muy afectada. La necesidad mayor de oxígeno era atendida por mayores entradas y salidas de aire en todas las enfermas, aparte de las incapacitadas más gravemente, por medio de un aumento de la ventilación obtenido por la aceleración de la frecuencia respiratoria.

La respuesta de la capacidad ventiladora y de los volúmenes pulmonares al embarazo fué idéntica en las mujeres normales y en las que tenían insuficiencia pulmonar moderada o grave. En los tres grupos, la capacidad respiratoria máxima y la capacidad vital fueron a término iguales que cuando no existía embarazo. Las subdivisiones de la capacidad pulmonar total de todas las pacientes mostraron las alteraciones que caracterizan al aumento de la presión intraabdominal: disminución de la reserva expiratoria y de los volúmenes residuales y leve aumento de la capacidad inspiratoria.

En contraposición a las mujeres normales, las que tenían moderada insuficiencia pulmonar no se quejaron de disnea en ninguna ocasión durante el embarazo. Las que tenían insuficiencia grave no estuvieron más incapacitadas durante la gestación que antes. Dedújose que las pacientes que tienen insuficiencia restrictiva de la ventilación y no muestran disnea en reposo deben tolerar el embarazo sin mucha dificultad. Estas conclusiones no rezan forzosamente con enfermas en quienes la insuficiencia debe se a hiperresistencia de las vías aéreas o a pérdida de la elasticidad pulmonar.

RESUME

Fonction Pulmonaire dans la Grossesse: III. Observations en Série de Malades Presentant une Insuffisance de la Fónction Pulmonaire

Sept malades ayant une fonction pulmonaire déficiente déterminée par la tuberculose, le collapsus ou la résection ont été observées au cours de neuf grossesses. La fonction pulmonaire a été étudiée avant, aussitôt après et longtemps après l'intervention chirurgicale. Les examens ont été répétés avant, pendant et après la gestation.

Les observations effectuées avant la grossesse ont révélé divers degrés d'insuffisance de la ventilation, de "modéré" à "sévère", (*restrictive*), une diminution de la capacité respiratoire maxima et une réduction symétrique des subdivisions de la capacité pulmonaire totale. Aucune lésion de caractère obstructif n'a été observée. Les volumes par minute, les rapports entre le volume résiduel et la capacité pulmonaire totale, de même que les indices des mélanges gazeux alvéolaires demeuraient dans les limites normales.

Les alterations fonctionnelles observées chez les tuberculeuses au terme de la grossesse ont été comparées à celles qui existaient chez 19 femmes gravides normales.

Chez les femmes non-tuberculeuses, on constatait une hyperventilation au repos et à l'effort. Cette tendance était moins marquée chez les malades dont l'insuffisance de la fonction pulmonaire était modérée et absente chez celles dont la fonction pulmonaire était déficiente. Les besoins en oxygène augmentés se traduisaient par un accroissement du volume de l'aire courant chez toutes les femmes, sauf les plus atteintes, qui augmentaient la ventilation par l'accélération du rythme de la respiration.

Pendant la gestation la réaction de la capacité de ventilation et de la capacité pulmonaire était semblable chez les sujets normaux et chez les malades ayant une déficience modérée ou marquée de la fonction pulmonaire. Dans les trois groupes, la capacité respiratorie maxima et la capacité vitale étaient les mêmes chez les femmes gravides à terme et chez les femmes non-gravides. Les subdivisions de la capacité pulmonaire totale chez toutes les malades présentaient les modifications caractéristiques de l'augmentation de la pression intra-abdominale; la réserve expiratoire et les volumes résiduels étaient diminués et la capacité inspiratoire légèrement augmentée.

Par contraste avec les sujets normaux, les malades ayant une insuffisance modérée de la fonction pulmonaire ne se plaignaient de dysponée a aucun moment de la gestation. Les malades chez lesquelles cette fonction était très déficiente n'étaient pas plus gênées pendant la grossesse qu'auparavant.

Sur la base des études présentées, la réserve repiratoire probable et l'indice de dyspnée chez la femme gravide a terme peuvent être calculées lorsque la capacité de la ventilation est connue—En conclusion, les malades présentant une insuffisance de ventilation "*restrictive*", sans être affectées de dyspnée au repos, doivent pouvoir supporter l'état de grossesse sans grande difficulté. Ces conclusions ne s'appliquent pas aux cas d'insuffisance de ventilation déterminée par une résistance se manifestant au niveau des voies aériennes ou par une perte d'élasticité du tissu pulmonaire.

Acknowledgment

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THE TOXICITY OF ISONIAZID FOR THE RHESUS MONKEY[1, 2]

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INTRODUCTION

For more than nine months the effects of isoniazid on naturally acquired tuberculosis in the rhesus monkey have been under study in this laboratory. Observations on the acute, subacute, and chronic toxicities of this drug have formed an integral part of the study. These observations have demonstrated conclusively that the monkey tolerates relatively large doses of isoniazid for protracted periods and exhibits no untoward reaction to doses which are reportedly lethal for the dog (1, 2). This demonstration may have significant implications for clinical use of isoniazid since it may encourage administration of larger and possibly more effective doses of this drug in human tuberculosis. The use of large doses of isoniazid in man has been approached with great conservatism, apparently because of the purportedly high toxicity of the compound for the dog. In view of these implications, a brief summary of the simian study seems indicated even though all phases of the work have not been completed.

EXPERIMENTAL

Methods

Acute toxicity: The acute oral toxicity of isoniazid[3] was studied in a group of 18 rhesus monkeys (*Macaca mulatta*). These animals, ranging in weight from 3.8 to 6.1 kg., were thoroughly adapted to the laboratory environment and were negative cutaneous reactors to tuberculin on repeated tests. They had been used for studies on the chemotherapy of primate malaria (infections with *Plasmodium cynomolgi*); however, all had been cured of this disease more than three months previously and were in excellent health at the beginning of the present study. Isoniazid was administered to the monkeys in single doses ranging from 80 to 320 mg. per kg. In all cases, the requisite amount of drug, dissolved in 50 ml. of water, was administered by stomach tube approximately sixteen hours after the last meal on the previous day. Animals were observed constantly for eight hours after treatment, then twice daily for fourteen additional days.

Subacute toxicity: Four rhesus monkeys, weighing 3.3 to 4.3 kg., were used in this study. The animals had been acclimated to the quarters for ten weeks, were tuberculin-negative, outwardly normal in every respect, and had not been used previously. Initially, isoniazid was administered in daily doses of 2.5 and 10 mg. per kg., 2 monkeys receiving each dosage. One half of the daily dose was given at 8 a.m., the other half at 4:30 p.m. As in the acute experiments, the drug was administered in aqueous solution by stomach tube. The smaller of the above doses was administered for fourteen consecutive days. In the absence of adverse reactions and on the basis of the acute toxicity data not available at the start of the study, the daily dose was increased to 40 mg. per kg. and maintained at this level for thirty-

[1] From The Christ Hospital Institute of Medical Research, Cincinnati, Ohio.

[2] This study was supported in part by a research grant from the National Institutes of Health, Public Health Service.

[3] We are indebted to the Wm. S. Merrell Company, Cincinnati, Ohio, for the isoniazid used throughout this study.

eight days, at which time both animals were sacrificed for histopathologic study. At this point, the 2 remaining monkeys had received 10 mg. per kg. doses for fifty-two days without untoward reactions. Daily intakes were then increased to 80 mg. per kg. and continued at this level for twenty days, after which the animals were sacrificed.

Throughout the above treatment periods, the monkeys were examined daily for evidences of hyperirritability or central nervous system dysfunction. Complete hematologic studies and icterus index and methemoglobin determinations were performed twice weekly; animals were weighed once weekly. Isoniazid concentrations in plasma were determined on the thirty-eighth day of the 10 mg. dosage regimen, the thirty-fourth day of the 40 mg., and the ninth and eighteenth days of the 80 mg. regimens. In each instance, analyses were performed on oxalated blood samples obtained just prior to the 8 a.m. treatment and one, two, four, and eight hours thereafter.

As noted previously, animals were sacrificed at the end of the 40 and 80 mg. treatment periods, at which times complete necropsies were performed. Specimens of thyroid, heart, aorta, lung, liver, spleen, pancreas, stomach, small and large intestines, kidney, ureter, bladder, adrenal, ovary, axillary and inguinal lymph nodes, and femoral bone marrow were fixed in Zenker-formalin, embedded in paraffin, sectioned, and stained with hematoxylin-eosin and Maximow. Smears of femoral marrow were stained with Wright and Giemsa. The brain and entire cord were removed and fixed in 10 per cent neutral formalin; these organs have not been sectioned and studied to date.

Chronic toxicity: This study, performed incidental to definition of the effects of isoniazid on simian tuberculosis, has involved work on 169 monkeys. At the beginning of treatment, 118 of these animals were tuberculin-positive and 51 were tuberculin-negative. All had been used previously in studies on the chemotherapy of malaria but had been cured of this disease at least three months prior to the present study. Approximately 25 per cent of the tuberculin-positive monkeys were seriously debilitated. On April 28, 1952, isoniazid was added to the basal diet[4] of these animals in a concentration which gave an average daily intake of approximately 5 mg. per kg. This fortified diet was fed for seventy-one days (until July 7, 1952), at which time 27 tuberculin-positive and 17 tuberculin-negative monkeys were sacrificed. For reasons not pertinent to this discussion, the concentration of isoniazid in the diet was then quadrupled to give a daily intake of approximately 20 mg. per kg. Nineteen tuberculin-positive and 3 tuberculin-negative monkeys were sacrificed after ninety days at this dosage. With four exceptions,[5] the remaining 72 tuberculin-positive and 31 tuberculin-negative animals are receiving the higher intake of isoniazid at the present date (February 9, 1953), the duration of the 20 mg. dosage period being two hundred and seventeen days.

The above monkeys have been observed daily for evidences of toxicity and have been weighed monthly or more frequently. Complete hematologic studies were carried out between December 8 and December 20, 1952, on 102 of these animals. The concentrations of isoniazid in the plasma of 16 representative monkeys were determined after thirty-eight days of treatment at 5 mg. per kg. and after eight, fifty-eight, one hundred and thirty-six, and two hundred and eleven days of treatment at 20 mg. per kg. On each occasion, blood samples were obtained just prior to the morning and afternoon meals, the theoretical trough periods for each treatment interval.

Complete necropsies were performed on the 63 animals which were sacrificed as well as on the 4 which succumbed for other reasons. Tissues were searched grossly for abnormali

[4] The Okatie Farms Primate Ration (3) was the basal diet used in this study. The appropriate amounts of isoniazid, dissolved in tap water, were added to this ration immediately prior to feeding. The dosages of isoniazid were adjusted monthly according to the weights of the animals.

[5] These four exceptions included 2 animals which died of type 33 pneumococcal meningitis, one which succumbed to sepsis incidental to an abortion, and one which died during recrudescence of a supposedly cured malarial infection.

ties which could be attributed to isoniazid. Microscopic sections are being prepared currently, but none have been studied to date.

The effects of isoniazid on the above animals have been controlled by simultaneous studies on 303 monkeys fed the basal diet. These animals, also discarded from malaria chemotherapy experiments, were all tuberculin-negative and in excellent condition at the start of the study.

Determination of isoniazid in plasma: The concentrations of isoniazid in plasma were determined by the method of Kelley and Poet (4) with a few minor modifications. The latter involved (*a*) use of ethylene dichloride[6] in place of diethyl ether in the extraction mixture, (*b*) substitution of graduated, round-bottom tubes for conical tubes in the hydrolysis procedure, and (*c*) extension of the hydrolysis period from forty-five to sixty minutes.

Substitution of ethylene dichloride for ether in the solvent mixture has four distinct advantages. First, ethylene dichloride is less volatile than ether and provides more stable volume relationships, particularly when centrifuged. Second, use of ethylene dichloride eliminates troublesome emulsions which invariably form with ether. Third, by eliminating emulsions greater yields of extract are obtained, thus increasing the over-all sensitivity of the analytical method and reducing the volume of plasma required for analysis.[7] Finally, the use of ethylene dichloride makes it possible to obtain quantitative recoveries (97.6 to 101.3 per cent) with plasma samples containing isoniazid in concentrations greater than 2.0 mg. per liter. Satisfactory recoveries were not obtained when ether was used in the solvent system.

Because of uneven heating, reproducible results could not be obtained at any concentration of isoniazid when the hydrolysis was performed in conical tubes. This difficulty was eliminated by use of round-bottom tubes fitted with glass bubble condensers.

A forty-five-minute hydrolysis period, as prescribed by Kelley and Poet, gave satisfactory results only when plasma concentrations of isoniazid did not exceed 1 mg. per liter. However, with a sixty-minute period of hydrolysis, isoniazid concentrations up to 40 mg. per liter could be determined quantitatively.

RESULTS

Acute toxicity: As shown in table 1, isoniazid produced convulsions in 4 of 10 monkeys which received doses of 80 or 160 mg. per kg. and either convulsions or preconvulsive reactions in 7 of 8 monkeys which received 240 or 320 mg. doses. These convulsions, clonic in type, followed a fairly uniform pattern. With one exception, they appeared within ninety to one hundred and twenty minutes after treatment and were preceded by a ten- to twenty-minute interval of mixed apprehension, excitement, and hyperirritability. The initial convulsion was usually the most severe and lasted five to fifteen minutes; this was generally followed by two or more convulsive episodes of shorter duration and lesser severity, recovery being complete within three hours of treatment.

There were two exceptions to this sequence of events. One animal, Monkey 4731, had especially severe convulsions only forty minutes after treatment and died during the third and most severe seizure. A second animal, Monkey 4771, had apparently recovered completely from the effects of isoniazid three hours after treatment; however, for another three hours convulsive episodes could be produced merely by chasing this monkey about the catching area.

[6] Prior to use in the extraction mixture, laboratory grade ethylene dichloride is shaken with vegetable charcoal (Darco) and then filtered through double layers of paper.

[7] With the modifications described it is possible to use 2 to 3 ml. quantities of plasma and to measure isoniazid concentrations as low as 0.04 mg. per L.; 3 ml. quantities of plasma were usually employed in the present study.

The convulsant action described above was the only outward manifestation of the acute toxicity of isoniazid. There were no delayed toxic effects during the two-week post-treatment observation period.

Subacute toxicity: The findings in this study were essentially negative and may be summarized very briefly. Administration of isoniazid at daily doses of 2.5 mg. per kg. for fourteen days, 10 mg. for fifty-two days, 40 mg. for thirty-eight days, or 80 mg. for twenty days evoked no outward manifestations of toxicity. Convulsive reactions or prodromal symptoms such as occurred in the acute study were entirely absent.

TABLE 1

THE ACUTE TOXICITY OF ISONIAZID

MONKEY NUMBER	DOSE OF ISONIAZID MG. PER KG. OF BODY WEIGHT	REACTIONS TO TREATMENT
4643	80	None
4797	80	None
4877	80	None
4689	80	Clonic convulsions at 120 minutes; complete recovery at 180 minutes
4789	80	Clonic convulsions at 90 minutes; complete recovery at 180 minutes
4799	160	None
4884	160	None
4894	160	None
4398	160	Clonic convulsions at 120 minutes; complete recovery at 180 minutes
4771	160	Clonic convulsions at 120 minutes; complete recovery at 390 minutes
4737	240	Apprehensive and hyperirritable at 120 minutes; recovery at 180 minutes
4768	240	Clonic convulsions at 105 minutes; complete recovery at 240 minutes
4808	240	Clonic convulsions at 105 minutes; complete recovery at 120 minutes
4731	240	Clonic convulsions at 40 minutes; death at 80 minutes
4779	320	None
4626	320	Ataxic at 120 minutes; complete recovery at 180 minutes
4778	320	Clonic convulsions at 110 minutes; complete recovery at 120 minutes
4706	320	Clonic convulsions at 115 minutes; complete recovery at 150 minutes

The above treatment caused no alterations in formed elements of peripheral blood or bone marrow and produced neither gross nor microscopic lesions in thyroid, heart, lung, aorta, liver, spleen, stomach, small or large intestine, pancreas, kidney, ureter, bladder, adrenal, ovary, or axillary and inguinal lymph nodes. Three of the four monkeys exhibited a slight weight gain such as would be anticipated in young healthy animals; the weight of the fourth monkey did not change.

Data on the concentrations of isoniazid in plasma, summarized in table 2, show clearly that the absence of toxic symptoms cannot be attributed to failure of absorption from the gastrointestinal tract. Significant concentrations of drug were attained at all doses, the relationship between dose and plasma concentrations being remarkably linear. The data, although limited, also suggest that plasma concentrations attained on a given dose are fairly reproducible in different animals.

TABLE 2

CONCENTRATIONS OF ISONIAZID IN PLASMA OF MONKEYS RECEIVING REPEATED DOSES

MONKEY NUMBER	DAILY DOSE MG. PER KG. OF BODY WEIGHT	DAY OF TREATMENT	MG. OF ISONIAZID PER LITER OF PLASMA				
			Hours after 8 a.m. Treatment				
			0*	1	2	4	8
4956	10	38	0.39	2.66	2.62	2.19	1.12
4965	10	38	0.77	4.08	3.93	2.93	1.27
4947r	40	34	2.43	15.21	13.67	10.24	4.80
4952r	40	34	2.73	16.56	14.63	9.86	4.93
4956r	80	9	4.24	26.18	lost	18.80	11.02
4956r	80	18	4.86	18.48	25.42	19.48	12.32
4965r	80	9	3.16	18.48	19.56	15.24	10.48
4965r	80	18	5.92	16.64	28.18	28.10	15.32

* Approximately fifteen hours after previous dose.

TABLE 3

EFFECTS OF PROTRACTED ADMINISTRATION OF ISONIAZID ON BODY WEIGHT

GROUP	ISONIAZID TREATMENT	NUMBER OF MONKEYS IN GROUP	KG. OF WEIGHT CHANGE AVERAGE PER MONTH OF OBSERVATION
Tuberculin-negative	None	280	+0.114
Tuberculin-negative	Daily doses of 5 mg. per kg. for two months followed by 20 mg. per kg. for seven months	29	+0.113
Tuberculin-positive	None; observed from 3-21-52 to 1-27-53 or death	23	−0.380
Tuberculin-positive	Daily doses of 5 mg. per kg. for two months followed by 20 mg. per kg. for seven months	69	+0.137

Chronic toxicity: Administration of isoniazid in daily doses of 5 mg. per kg. for two months followed by treatment with 20 mg. per kg. doses for seven months evoked no outward manifestations of toxicity. On the contrary, there was marked and nearly uniform improvement in the general physical condition and activity of tuberculin-positive animals, many of which were severely debilitated at the start of treatment. The fact that 15 monkeys have become pregnant and that 4 of these have already delivered healthy offspring is a good indication of the excellent condition of the colony. As shown in table 3, both tuberculin-positive and

tuberculin-negative monkeys receiving isoniazid exhibited weight gains comparable to those of untreated tuberculin-negative animals and strikingly different from the weight losses of untreated tuberculin-positive monkeys.

Hematologic studies carried out on 102 monkeys which received isoniazid as described above have revealed no adverse effects of this treatment on either the

TABLE 4

EFFECTS OF PROTRACTED ADMINISTRATION OF ISONIAZID ON FORMED ELEMENTS OF PERIPHERAL BLOOD

HEMATOLOGIC COMPONENT	MEAN FOR 102 MONKEYS TREATED WITH ISONIAZID ± STANDARD DEVIATION	AVERAGES REPORTED IN LITERATURE	
		Scarborough (5)	Sundberg, Schaar, and May (6)
Erythrocytes × 10^6 per cu. mm.	5.13 ± 0.50	5.59	6.25
Hemoglobin gm. per 100 ml.	11.09 ± 1.38	—	12.71
Leukocytes × 10^3 per cu. mm.	12.73 ± 2.26	16.2	15.4
Per cent mature neutrophils	46.19 ± 14.87	—	—
Per cent total neutrophils	46.59 ± 14.77	42.2	49.05
Per cent lymphocytes	43.33 ± 12.67	52.8	44.05
Per cent monocytes	3.68 ± 1.99	1.2	2.88
Per cent eosinophils	5.89 ± 4.07	3.7	3.28
Per cent basophils	0.63 (range 0.0 to 3.0)	0.52	0.74

TABLE 5

"TROUGH" CONCENTRATIONS OF ISONIAZID IN PLASMA OF MONKEYS RECEIVING PROTRACTED TREATMENT

PERIOD OF EXAMINATION	MG. OF ISONIAZID PER LITER OF PLASMA ± STANDARD DEVIATION				
	6-14-52‡	7-15-52§	9-3-52§	11-20-52§	2-3-52§
Morning*	0.129 ± 0.04	1.13 ± 0.447	0.615 ± 0.326	0.676 ± 0.375	0.75 ± 0.322
Afternoon†	0.192 ± 0.085	1.554 ± 0.295	1.352 ± 0.609	1.63 ± 0.373	1.60 ± 0.401

* Sixteen hours after afternoon meal of preceding day and immediately prior to morning meal.

† Eight hours after morning meal and immediately prior to afternoon meal.

‡ Determinations performed after thirty-eight days of treatment with doses of 5 mg. per kg.

§ Determinations performed after eight, fifty-eight, one hundred and thirty-six, and two hundred and eleven days of treatment with doses of 20 mg. per kg.

numbers or morphology of circulating erythrocytes and leukocytes. In every significant respect, the data acquired on treated animals (table 4) are comparable to those on normal rhesus monkeys reported from other laboratories (5, 6) and to findings on more than 2,000 normal monkeys which have been studied in this laboratory during the past twelve years (7).

As shown by the data in table 5, significant concentrations of isoniazid were maintained in plasma throughout the treatment intervals. The concentrations

attained on the 20 mg. dosage schedule were far greater, however, than those on the 5 mg. regimen. At the higher dosage, the mean plasma concentrations ranged from 0.615 to 1.13 mg. per liter at the morning "trough period" and from 1.35 to 1.63 mg. per liter at the afternoon "trough period." It is significant that the concentrations of isoniazid at these respective intervals did not change significantly during the course of treatment, a finding which supports the conclusion that, at doses of 20 mg. per kg., the drug does not accumulate in the animal body.

Gross examination of the organs and tissues of the above monkeys revealed no reaction or lesion which could be attributed to isoniazid. It is especially noteworthy that livers and kidneys were entirely normal.

DISCUSSION

The studies described above have demonstrated that isoniazid evokes no adverse reactions in the rhesus monkey when administered for more than seven months in divided daily doses of 20 mg. per kg. There is a suggestion, at least, that even larger doses would be tolerated by this animal since divided daily doses of 40 and 80 mg. per kg. evoke no toxicity when administered for thirty-eight and twenty days, respectively, and since isoniazid appears to have little or no cumulative toxicity. Daily doses larger than 80 mg. per kg. would probably not be tolerated unless administered in more than two fractions, for in acute experiments single doses of 80 mg. per kg. and larger provoked severe convulsions.

This convulsant action and associated prodromal symptoms were the only manifestations of isoniazid toxicity in either acute or chronic studies.

The complete absence of untoward effects on the hematopoietic system, liver, kidneys, digestive tract, and endocrine organs is certainly worthy of emphasis.

As mentioned previously, these findings in the rhesus monkey have been set forth largely because of their striking contrast to the reported toxicity of isoniazid for the dog (1, 2). In the latter animal, single doses of 50 mg. per kg. produced fatal convulsions regularly; divided daily doses of 17.5 to 25 mg. per kg. were lethal within a period of twenty-eight to thirty-three days. Moreover, the last-named doses were reported to have produced hepatic and renal injury, anemia, and depletion of the erythroid elements of bone marrow. Whereas the evidence presented in support of organ pathology is not too convincing, there can be little question that in the over-all picture isoniazid is considerably more toxic for the dog than for the monkey.

This difference in toxicity of isoniazid for the dog and monkey is of more than academic interest, for the dog data have been assumed to be a valid indicator of the tolerability of isoniazid in man, have guided initial choice of dosage in human therapeutics, and have seemingly discouraged thorough explorations of the toxicity and dose-therapeutic effect relationships in man.

One could not criticize these practices if the original assumption rested on a sound basis. Unfortunately, that is not true, for there are many situations in which the toxicity of a drug for the dog provides little indication of what may be expected in the human subject. Three groups of antimalarial drugs which the present writers have studied extensively illustrate this point clearly. Thus, doses

of quinine which are used routinely in human malaria produce optic atrophy and blindness in the dog (8). Doses of chloroquine which are well tolerated in man (9) produce fatal convulsions in the dog (10). Certain 8-aminoquinolines which produce severe injury to localized areas of the central nervous system of man evoke neither functional nor anatomic evidence of such injury in the dog (10–12). Other 8-aminoquinolines which in humans have destructive effects on the formed elements of peripheral blood and bone marrow are devoid of such action in the dog (10, 12). In each of these instances, the reactions of the rhesus monkey are reliable indicators, both qualitative and quantitative, of the reactions of the human subject (8, 10–12).

Many other instances of the diverse reactions of man and dog to the same drug could be cited. It is especially pertinent to note, however, that semicarbazide, a compound closely related chemically to isoniazid and a similar pharmacologic convulsant, is at least four times as toxic for the dog as for man and is approximately six times as toxic for the dog as for the monkey (13). This throws special weight against acceptance of data on the toxicity of isoniazid for the dog as a valid indicator of human tolerability.

It is hoped that the above comments and particularly the results of the monkey toxicity studies reported here will stimulate systematic explorations of the reactions of the human subject to higher doses of isoniazid than are being used routinely at present. Actually, available human experience indicates the need for restudy and reappraisal of the human tolerance for this drug. Klee (14) administered doses of 10 to 15 mg. per kg. to 61 patients for six weeks and longer without untoward reactions. Clark and co-workers (15) administered doses of 10 mg. per kg. to 25 patients for one week without adverse effects, while Biehl (14) administered 15 mg. per kg. doses to one individual for more than five months, again without toxic reactions. Selikoff and colleagues (17) have suggested "that 20 mg. per kg. doses may prove excessively toxic" but have not presented the evidence on which this suggestion is based. None of the toxic effects of isoniazid in man recorded to date would militate against thorough explorations of human tolerance for higher doses.[5] Such studies will be of vital importance if, as seems likely, routine administration of isoniazid in doses larger than the 3 to 5 mg. per kg. regimen in current use proves necessary for exploitation of the full potentialities of this compound in the therapy of human tuberculosis.

SUMMARY

Studies on the toxicity of isoniazid for the rhesus monkey have shown that this drug produces no adverse reactions when administered in divided daily

[5] Two preliminary reports bearing on the tolerability of isoniazid in the human subject have appeared since this manuscript was submitted for publication. Both deal with treatment of tuberculous meningitis or miliary tuberculosis in infants. In one (18) isoniazid was administered at daily doses of 8 to 16 mg. per kg. for three to four months without adverse effects. In the second (19), the drug was administered in daily doses of 10 to 20 mg. per kg. to patients in the Zurich Kinderspital and in doses up to 40 mg. per kg. elsewhere, without significant side reactions.

doses of 20 mg. per kg. for more than seven months, or in divided daily doses of 40 and 80 mg. per kg. for thirty-eight and twenty days, respectively. The occurrence of convulsions following single oral doses of 80 to 320 mg. per kg. is the only toxic effect noted to date. The complete absence of tissue toxicity of isoniazid is especially notable.

The relatively low toxicity of isoniazid for the monkey, and particularly the absence of injury to various organ systems, is in striking contrast to reported effects of this drug on the dog, effects which to this point have been a major consideration in limiting the dosage in human subjects. In view of the findings in the monkey and the repeated disparity between the reactions of man and dog to many drugs, it is suggested that it would be highly desirable to initiate a cautious but systematic appraisal in the human subject of the toxicologic liabilities of substantially larger doses of isoniazid than are being used currently in the treatment of tuberculosis.

SUMARIO

La Toxicidad de la Isoniacida para el Mono Rhesus

Los estudios de la toxicidad de la isoniacida para el mono rhesus han demostrado que esa droga no produce reacciones adversas cuando se administra a dosis diarias fraccionadas de 20 mg. por kg. durante más de siete meses o a dosis diarias fraccionadas de 40 y 80 mg. por kg. durante treinta y ocho y veinte días, respectivamente. La ocurrencia de convulsiones después de dosis orales únicas de 80 a 320 mg. por kg. es el único efecto tóxico notado hasta la fecha. En particular es notable la absoluta ausencia de toxicidad de la isoniacida para los tejidos.

La toxicidad relativamente baja de la isoniacida para el mono, y en particular la falta de nocividad para varios sistemas de órganos, forma un notable contraste con los efectos de la droga comunicados en el perro, efectos esos que hasta ahora han constituído un factor principalísimo en la limitación de la dosis en sujetos humanos. En vista de los hallazgos en el mono y la repetida discrepancia entre las reacciones del hombre y del perro a muchas drogas, sugiérese lo convenientísimo que sería iniciar una justipreciación cautelosa, pero sistemática, en seres humanos de los riesgos toxicológicos que entrañarían dosis substancialmente mayores de isoniacida que las usadas actualmente en el tratamiento de la tuberculosis.

RESUME

La toxicité de l'Isoniazide pour le singe Rhésus

Les recherches sur la toxicité de l'isoniazide chez le singe rhésus ont montré que ce médicament ne produisait aucune réaction nocive quand il est administré à la dose quotidienne fractionnée de 20 mg. par kg. pendant plus de sept mois, ou à la dose quotidienne fractionnée de 40 et de 80 mg. par kg. pendant trente-huit et vingt jours, respectivement. L'apparition de convulsions à la suite de doses orales uniques de 80 à 320 mg. par kg. est le seul effet toxique qui ait été observé jusqu'à présent. L'absence complète de toxicité de l'isoniazide pour les tissus est particulièrement remarquable.

La toxicité relativement basse de l'isoniazide pour le singe, en particulier l'absence de lésions sur les divers systèmes organiques, est en opposition saisissante avec les effets produits par ce médicament sur le chien, effets qui, jusqu'à maintenant, ont été une des raisons majeures de la limitation des doses chez l'homme. En raison des résultats obtenus chez le singe et des différences fréquemment observées entre les réactions de l'homme et du chien à de nombreux médicaments, il est suggéré qu'il serait particulièrement désirable d'entreprendre chez l'homme, avec prudence, mais d'une façon systématique, l'évaluation

des risques toxicologiques de doses d'isoniazide nettement plus élevées que celles utilisées couramment dans le traitement de la tuberculose.

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IN VITRO AND IN VIVO ACTIVITY OF STREPTOMYCIN AND ISONIAZID SINGLY AND IN COMBINATION[1, 2]

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INTRODUCTION

The observations on the action of streptomycin and para-aminosalicylic acid (PAS), made in recent years, have indicated the value of utilizing combinations of antimicrobial agents for the treatment of certain infectious diseases such as tuberculosis. Indeed, it has become apparent that even a weak tuberculostatic agent such as PAS, when used in combination with streptomycin, may serve to prevent the emergence of streptomycin-resistant organisms. The introduction of isoniazid (1–4), a substance comparable to streptomycin in degree of activity against the tubercle bacillus, suggested the possibility that streptomycin and isoniazid might be used together even more advantageously than was the case with streptomycin and PAS.

The present study was undertaken to evaluate the action of streptomycin and isoniazid singly and in combination.

MATERIALS AND METHODS

Highly purified streptomycin sulfate, prepared through a crystalline stage, and crystalline isoniazid were used throughout the study. Streptomycylidene isonicotinyl hydrazine[3, 4] was prepared by chemical combination of these two substances and was crystalline in nature (5).

[1] From the Research Laboratories of Chas. Pfizer & Co., Inc., Brooklyn 6, New York.

[2] Presented in part at the annual meeting of the American Public Health Association, Cleveland, Ohio, October 24, 1952.

[3] Streptomycin and isoniazid may be combined in molecular proportions to form a crystalline compound. This compound, which is streptomycylidene isonicotinyl hydrazine, will be described in a forthcoming publication by Pennington and associates (5). Each 1 gm. of streptomycin combines with 236 mg. of isoniazid. Fifty per cent aqueous solutions of streptomycylidene isonicotinyl hydrazine at room temperature reach an equilibrium within approximately ten minutes. When equilibrium is reached, approximately 95 per cent of the dissolved content is in the form of unhydrolyzed streptomycylidene isonicotinyl hydrazine, while approximately 5 per cent is in the form of a mechanical mixture of streptomycin and isoniazid. Fifty per cent aqueous solutions of streptomycin and isoniazid, when mixed mechanically in the same ratio as would be used for preparation of streptomycylidene isonicotinyl hydrazine, also reach an equilibrium within approximately ten minutes, at which time approximately 95 per cent of the dissolved content again is in the form of unhydrolyzed streptomycylidene isonicotinyl hydrazine and the remainder in the form of a mechanical mixture of streptomycin base and isoniazid. The dissolved content of 1.0, 0.1, 0.01, and 0.001 per cent aqueous solutions of streptomycylidene isonicotinyl hydrazine, at room temperature—when equilibrium has been reached, consists of 20, 50, 85, and 98 per cent of free streptomycin base and isoniazid, respectively; while 80, 50, 15, and 2 per cent, respectively, is in the unhydrolyzed form. The writers are indebted to Dr. Pennington for all streptomycylidene isonicotinyl hydrazine used in this study.

[4] Streptohydrazid is the proprietary name of Chas. Pfizer & Co., Inc., for streptomycylidene isonicotinyl hydrazine.

The methods used for the evaluation of streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine have been described in detail elsewhere (6-8). A liquid Tween[5]-albumin medium was used for all *in vitro* sensitivity determinations. The conventional pour-plate technique for estimation of the number of clumps of viable bacilli in a culture at any given period served as an aid in the evaluation of the tuberculostatic or tuberculocidal action of these agents. For this phase of the study, a transparent oleic-acid-albumin solid medium was utilized. The counts obtained by this method, in the experience in the laboratory, are in close agreement with those obtained by the method of Fenner, Martin, and Pierce (9). Transfers to determine the rate of emergence of drug-resistant organisms were made on Szybalski gradient plates, using the oleic-acid-albumin solid medium (10).

In vivo studies of the action of streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine were performed in both guinea pigs and mice (Webster Swiss strain male white mice, 20 gm. weight). The H37Rv strain of *M. tuberculosis* var. *hominis*, was used throughout all studies conducted in guinea pigs, while the Vallée strain[6] of *M. tuberculosis* (bovine) and the intravenous route of inoculation were used in all mouse experiments.

Unless otherwise stated, treatment was started in all instances within twenty-four hours after infection and was continued until the day preceding autopsy. Two procedures were utilized in those studies conducted in an attempt to demonstrate the presence of viable tubercle bacilli in the organs of drug-treated animals:

(*a*) direct inoculation of macerated tissue into a liquid Tween-albumin medium, and

(*b*) subcutaneous injection into guinea pigs of 0.2 ml. of tissue homogenates prepared by homogenizing the entire lung (or spleen) in 5 ml. of physiologic saline.

RESULTS

In Vitro Activity

Susceptibility to streptomycin, isoniazid, and streptomycylidene isonicotinyl hydrazine: Preliminary experiments were conducted to determine the susceptibility of a number of strains of *M. tuberculosis* (human, bovine, and avian) to streptomycin, isoniazid, and (for comparison) viomycin, a third potent tuberculostatic agent. As illustrated in table 1, isoniazid, like streptomycin, is highly active against both human and bovine strains of tubercle bacilli not previously in contact with this antituberculous agent. Based on the limited number of strains tested, it is further apparent that isoniazid is active against both streptomycin-susceptible and streptomycin-resistant organisms and against certain viomycin-susceptible and viomycin-resistant strains, while streptomycin is effective against both isoniazid-susceptible and isoniazid-resistant strains.

The susceptibilities of three strains of *M. tuberculosis* to isoniazid were determined in a medium containing amounts of streptomycin equivalent to one-fifth to one-fortieth of the minimal inhibitory concentration required for most susceptible organisms. In like manner, the susceptibilities of these strains to streptomycin were determined in a medium containing comparable amounts of isoniazid. The concentrations were selected in view of the fact that previous studies, conducted with *M. tuberculosis* and with other species of microorganisms, had

[5] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtained from the Atlas Powder Co., Wilmington, Delaware.

[6] Received through the courtesy of Dr. Cynthia Pierce of the Rockefeller Institute for Medical Research.

indicated that synergism[7] most frequently can be revealed in these concentrations (11). Using such concentrations, however, it was not possible to demonstrate readily a synergistic effect between streptomycin and isoniazid, although there also was no evidence of an antagonistic effect.

The observation by Pennington and his associates (5) to the effect that streptomycin and isoniazid may be combined in molecular proportions to form streptomycylidene isonicotinyl hydrazine suggested that this compound might be more active as a tuberculostatic agent than either streptomycin or isoniazid alone. Experiments were conducted to evaluate its action against *M. tuberculosis* in contrast to that of streptomycin and isoniazid alone. As shown in table 2, the total weight of streptomycylidene isonicotinyl hydrazine necessary for inhibition of growth was generally as great as the equivalent weight of strepto-

TABLE 1

COMPARISON OF THE ACTIVITY OF ISONIAZID, STREPTOMYCIN, AND VIOMYCIN (*In Vitro*)

STRAIN OF *M. tuberculosis*	SUSCEPTIBILITY: γ PER ML.*		
	SM	INH	VM
H37Rv (Bloch)	0.08	0.1	0.4
H37Rv (Steenken: mutant)	>760	0.1	0.4
Human isolates			
Munk. (VMR)	0.08	0.1	80.0
Mos	0.08	0.1	0.5
Kirchberg (avian)		>100	
Vallée (bovine)		0.4	
H37Rv (Bloch: mutant)†	0.2	12.5	
Vallée (mutant)†	<0.1	12.5	

* Susceptibility to isoniazid (INH) is expressed in terms of total weight; susceptibility to streptomycin (SM) and to viomycin (VM) is expressed in terms of the equivalent numbers of γ of pure streptomycin or viomycin base.

† Isoniazid-resistant mutants obtained by serial transfer of these strains in the presence of isoniazid.

mycin base required for such growth inhibition, except with streptomycin-resistant strains. If the weights of streptomycin and of isoniazid present in those concentrations of streptomycylidene isonicotinyl hydrazine necessary for inhibition of growth are compared with the minimal inhibitory concentrations of streptomycin alone and of isoniazid alone, it becomes apparent that streptomycin and isoniazid are readily effective when used in combination. There is no evidence, based on the results obtained in these experiments, to indicate that these two antituberculous agents antagonize one another.

Tuberculostatic and tuberculocidal action: The high antituberculous activity of isoniazid suggested the possibility that this agent, either alone or in combina-

[7] Throughout this report, the word "synergism" is used to refer to the concept of two agents acting together to produce an effect greater than that which could be achieved by the same quantity of each agent alone.

tion with streptomycin, might exert a tuberculocidal rather than a tuberculostatic effect *in vitro*. *M. tuberculosis*, strain H37Rv, and *M. butyricum* thus were grown in the presence of varying concentrations of isoniazid, streptomycin, and streptomycylidene isonicotinyl hydrazine, and the number of viable clumps per milliliter were determined at various time intervals. The results are illustrated in figures 1 and 2.

TABLE 2

TUBERCULOSTATIC ACTIVITY OF STREPTOMYCIN, IZONIAZID, AND STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE

(*In Vitro*)

STRAIN OF M. TUBERCULOSIS	SUSCEPTIBILITY: γ PER ML.*				
	Streptomycylidene Isonicotinyl Hydrazine†			INH	SM
	SIH	INH	SM		
H37Rv (Bloch)	0.40	0.07	0.28	0.10	0.40
	0.25	0.04	0.18	0.10	0.23
	0.63	0.10	0.44	0.12	0.40
Average	0.43	0.07	0.30	0.11	0.34
H37Rv (Steenken)	2.50	0.40	1.75	0.31	3,800
H37Rv (1075 St)	0.40	0.07	0.28		>1,000
	0.40	0.07	0.28	0.10	>1,000
	0.63	0.10	0.44	0.06	>3,800
Average	0.48	0.08	0.34	0.08	>1,000
Cummins (human)	0.25	0.04	0.18	0.10	0.15
	0.40	0.07	0.28	0.10	0.63
Average	0.32	0.05	0.23	0.10	0.39

* Concentrations of isoniazid and of streptomycylidene isonicotinyl hydrazine are expressed in terms of total weight. Concentrations of streptomycin are expressed in terms of the equivalent weights of streptomycin base.

† Streptomycylidene isonicotinyl hydrazine as the hydrochloride: 70 per cent streptomycin, 16 per cent isoniazid, by weight; complete hydrolysis assumed.

With *M. tuberculosis* (H37Rv), 0.1 γ of isoniazid per ml. was sufficient to produce a temporary bacteriostatic effect, while the equivalent of 0.1 γ of streptomycin base was incapable of altering the growth of the organism. In like manner, 0.1 γ of streptomycylidene isonicotinyl hydrazine, containing the equivalent of 0.07 γ of streptomycin base and 0.016 γ of isoniazid, per ml. of medium was insufficient to cause an inhibition of growth. No evidence of an additive or synergistic effect was apparent. One to 10 γ of streptomycin, isoniazid, or streptomycylidene isonicotinyl hydrazine, on the other hand, were sufficient to produce a marked decrease in the number of viable clumps.

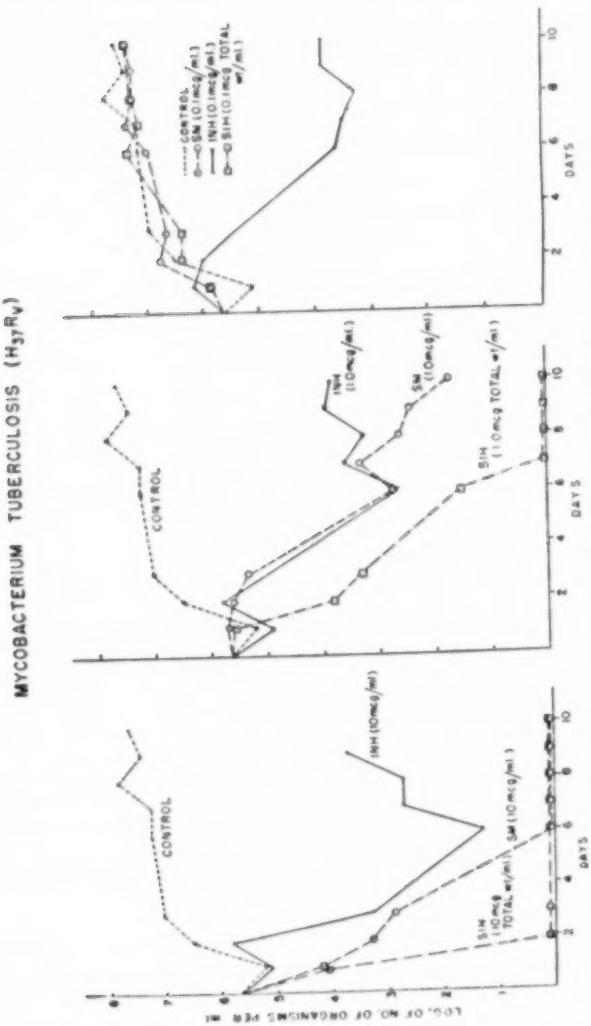

FIG. 1. The tuberculocidal action of streptomycin and isoniazid when used in combination

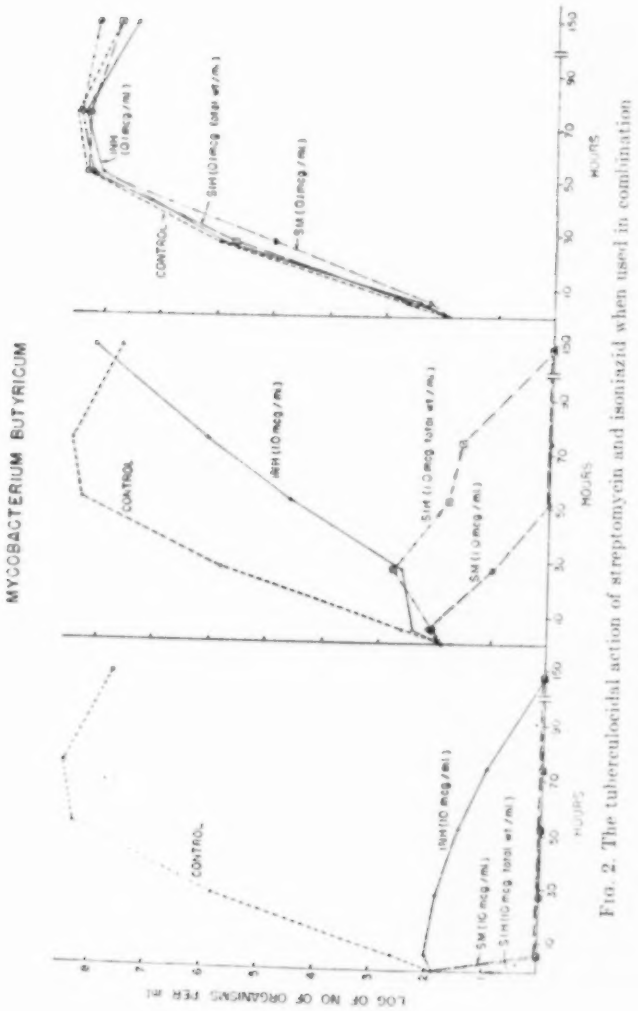

Fig. 2. The tuberculocidal action of streptomycin and isoniazid when used in combination

As illustrated in figure 1, when these high concentrations were used, it became apparent that:

(*a*) Streptomycin exerts as great, and possibly greater, tuberculocidal effect than isoniazid;

(*b*) The tuberculostatic or tuberculocidal action of isoniazid against this single human strain of *M. tuberculosis*, in contrast to that of streptomycin, frequently is temporary and is followed by an increase in the number of viable clumps;

(*c*) When streptomycylidene isonicotinyl hydrazine is used in concentrations (1 to 10 γ per ml.) high enough to contain amounts of streptomycin sufficient to produce a marked decrease in the number of viable clumps, or an actual elimination of all viable clumps, then the minimally effective or subinhibitory amounts of isoniazid present in this preparation are sufficient to produce an increase in the rate of killing of the organisms.

With *M. butyricum*, 0.1 γ of each of these three agents per ml. of medium was insufficient to alter the growth of the organism (figure 2). The equivalent of 1 to 10 γ of streptomycin base per ml., on the other hand, was sufficient to bring about a tuberculocidal effect and, indeed, these concentrations of streptomycin were more tuberculocidal than comparable amounts of isoniazid. One to 10 γ of streptomycylidene isonicotinyl hydrazine, or the equivalent of 0.7 to 7.0 γ of streptomycin base and 0.16 to 1.6 γ of isoniazid, respectively, per ml. of medium exerted an effect similar to that which resulted with comparable amounts of streptomycin alone. The minimal and subinhibitory amounts of isoniazid present in no way interfered with the action of the streptomycin.

Comparative rates of emergence of resistance: Preliminary studies by Hobby and Lenert (12) and by Middlebrook (13) have indicated that initially susceptible cultures of *M. tuberculosis* which have had no previous contact with isoniazid frequently contain large numbers of isoniazid-resistant cells. The fact that an apparent increase in the resistance of a given strain to isoniazid occurs rapidly when the strain is maintained in the presence of the antimicrobial agent also has been reported (14–18), and it is well known that strains resistant to streptomycin emerge rapidly after contact with the drug. The knowledge that para-aminosalicylic acid, a weak tuberculostatic agent, will suppress the emergence of streptomycin-resistant organisms when the two drugs are used in combination has led to the assumption by many that the combined use of streptomycin and isoniazid might delay the emergence of organisms resistant to each, and it seemed important to evaluate the validity of this assumption.

The rates of emergence of cells resistant to streptomycin, to isoniazid, and to streptomycylidene isonicotinyl hydrazine were compared when strains of *Mycobacteria* were grown in the presence of each. The results obtained with a single strain of *M. butyricum* and with a strain of *M. ranae* indicate, as shown in table 3, that there is a definite decrease in the rate of emergence of resistance to streptomycylidene isonicotinyl hydrazine in contrast to streptomycin or isoniazid alone. A hundred fold increase in resistance occurred after four to five transfers in the case of streptomycin, after five to six in the case of isoniazid,

and after nine to sixteen in the case of streptomycylidene isonicotinyl hydrazine. With the two saprophytic strains used for these studies, organisms made resistant to streptomycylidene isonicotinyl hydrazine were fully resistant to streptomycin alone and to isoniazid alone.

In a single experiment with *M. butyricum*, in which the susceptibility of those organisms used for each transfer was determined both to streptomycin and to isoniazid, there was a gradual stepwise increase in resistance to each of these agents through the first eight to nine transfers. Thereafter, there was a marked

TABLE 3

RATE OF EMERGENCE OF RESISTANCE TO STREPTOMYCIN, ISONIAZID, AND STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE

(*In Vitro*)

NUMBER OF TRANSFERS	SUSCEPTIBILITY: γ PER ML.*					
	M. butyricum			*M. ranae*		
	SIH	INH	SM	SIH	INH	SM
1	0.9	10	0.7	0.8	28	0.6
2	0.2	230	2.3	1.0		0.7
3	0.8	415	>152.0	3.5	545	1.5
4	1.0	905	>760.0	11.0	820	28.0
5	2.4	990		21.0	2,000	
6	4.0	1,110		26.0		213.0
7	5.5	1,150		31.0		760.0
8	7.0	2,000		28.0		
10	11.0			720.0		
11	37.0			1,000.0		
16	100.0					
19	245.0					
20	1,000.0					

* Concentrations of isoniazid (INH) and streptomycylidene isonicotinyl hydrazine (SIH) are expressed in terms of total weight; concentrations of streptomycin are expressed in terms of equivalent weights of streptomycin base.

increase in the rate of emergence of resistance to these agents. On the tenth transfer in the presence of streptomycylidene isonicotinyl hydrazine, the resistance of the organisms to streptomycin increased more than thirty-seven fold (from 27 to >1,000 γ per ml.), while the resistance of these organisms to isoniazid remained unaltered. On the thirteenth transfer, the resistance to isoniazid increased tenfold over that observed on the eighth to ninth transfers. Thus, there is a suggestion that the second-stage rapid development in resistance may possibly occur somewhat sooner with streptomycin than with isoniazid. Further studies are necessary to confirm this observation and to indicate whether or not the difference is significant.

Due to the obvious problems associated with the slow rate of growth of human strains of *M. tuberculosis*, demonstration of differences in the rate of emergence of resistance to streptomycin, isoniazid, or streptomycylidene isonicotinyl hy-

drazine has been more difficult. Nevertheless, preliminary observations on four strains of *M. tuberculosis* var. *hominis* are reported in table 4. With the single streptomycin- and isoniazid-susceptible strain used in this experiment, emergence of resistance to streptomycylidene isonicotinyl hydrazine occurred more slowly than to streptomycin or isoniazid. With a streptomycin-resistant strain and with a strain which became highly resistant to streptomycin on the second transfer in its presence, emergence of resistance to isoniazid and to streptomycylidene

TABLE 4

RATE OF EMERGENCE OF RESISTANCE TO STREPTOMYCIN, ISONIAZID, AND STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE

(*M. tuberculosis* var. *hominis*)

NUMBER OF TRANSFERS	SUSCEPTIBILITY: γ PER ML.*											
	Strain of Microorganism											
	H37Rv (Bloch)			Stiles			1075-ST†			INH-R‡		
	SIH	INH	SM	SIH	INH	SM	SIH	INH	SM	SIH	INH	SM
1	0.3	0.1	0.3	0.3	0.06	>0.8–<8.0	0.1	0.03	>1,600	0.1	100	0.08
2	0.4	0.1	0.4	0.1	0.06	>800	0.1	0.1		0.3		0.2
3		0.1	0.8	0.3	0.1		0.1	0.09		0.1	100	0.3
4	0.2	0.1	0.8		0.1		0.4	0.5				0.2
5	0.3	0.1		0.2	0.09		0.4	1.0		0.5	83	0.3
6	0.4	0.1	2.4	0.4	0.1		0.5	1.0		2.4	100	0.6
7	0.6	1.0	4.9	0.3	0.3		0.4	0.6		1.0	100	0.8
8	1.0	8.4	80.0	0.8	0.04		0.3			1.0	100	0.8
9	0.6	100.0		0.6						0.6	100	0.8
10		72.2									100	0.8
11											100	0.8

* Concentrations of isoniazid (INH) and streptomycylidene isonicotinyl hydrazine (SIH) are expressed in terms of total weight; concentrations of streptomycin are expressed in terms of equivalent weights of streptomycin base.

† Streptomycin- and streptothricin-resistant mutant of strain H37Rv.

‡ Isoniazid-resistant mutant of strain H37Rv (Bloch).

isonicotinyl hydrazine was not observed. In like manner, with a single isoniazid-resistant strain, emergence of resistance to streptomycin and to streptomycylidene isonicotinyl hydrazine was not noted during the observation period. Further studies are necessary to confirm these observations and to prove whether or not emergence of resistance to isoniazid or to streptomycin actually occurs more slowly with strains resistant to streptomycin or isoniazid, respectively.

In Vivo Activity

Chemotherapeutic action: Demonstration of the synergistic action of two highly active compounds such as streptomycin and isoniazid is generally more difficult *in vivo* than *in vitro*, for each is readily effective when used alone, even in low dosage. Nevertheless, studies to determine the chemotherapeutic action

of streptomycin, of isoniazid, of mixtures of these two compounds, and of streptomycylidene isonicotinyl hydrazine against tuberculous infections were carried out in both mice and guinea pigs. The experiments were organized in an attempt to observe a difference between combined therapy and treatment with one of these agents alone. In an acute and highly fatal tuberculous infection in mice, produced by the Vallée (bovine) strain of *M. tuberculosis*, 0.1 mg. of

TABLE 5

THE CHEMOTHERAPEUTIC ACTION OF ISONIAZID IN MICE

(*M. tuberculosis*, Strain Vallée)

DAILY DOSAGE* (MG. INH) SUBCUTANEOUS	NUMBER OF MICE	PER CENT SURVIVAL (42 DAYS)	LUNG CULTURES FOR TUBERCLE BACILLI‡: NUMBER POSITIVE/NUMBER CULTURED
1.0	10	100	0/10
0.5	10	100	1/10
0.1	10	100	1/10
0.0†	10	20	10/10
0.0	10	10	8/9

* Dosage of isoniazid (INH) is expressed in terms of total weight of drug.

† Saline 0.2 ml. daily administered to this series of animals.

‡ Sacrificed on forty-second day following infection and cultured at time of autopsy.

TABLE 6

THE CHEMOTHERAPEUTIC ACTION OF ISONIAZID IN EXPERIMENTAL TUBERCULOSIS OF GUINEA PIGS

(Strain H37Rv)

DAILY DOSAGE (MG./KG.)*	NUMBER OF ANIMALS	PER CENT SURVIVAL (104 DAYS)	LUNG CULTURES FOR TUBERCLE BACILLI: NUMBER POSITIVE/ NUMBER CULTURED (SACRIFICED 104 DAYS)
5.0	10	100	0/10
2.5	9	90	0/9
1.0	7	100	0/7
0	9	77	8/9

* Subcutaneous therapy: Therapy started twenty-four hours after infection. Drug given in two daily doses, one every twelve hours. Dosage expressed in terms of total weight of drug.

isoniazid per day was sufficient to protect 100 per cent of animals. Cultivation of lung tissue from treated animals in a liquid Tween-albumin medium rarely showed evidence of viable organisms (table 5).

In a more slowly progressing infection of guinea pigs, produced by the H37Rv strain of *M. tuberculosis*, similar results were obtained (table 6). Treatment was started in these animals twenty-four hours after infection and before the allergic reaction had had time to develop. The results thus indicate only that isoniazid

prevents development of the disease in artificially inoculated guinea pigs. That survival time is not indicative of the degree of tuberculosis in this species of animal is recognized and, indeed, has not been accepted as a criterion of degree of infection. All animals were observed clinically and at post-mortem examination for extent of disease and, for the purposes of the record, were photographed at the time of autopsy. Histopathologic studies uniformly indicated failure of the disease to develop in isoniazid-treated animals.[8]

The chemotherapeutic action of mixtures containing varying proportions of streptomycin and isoniazid and the action of streptomycylidene isonicotinyl hydrazine were compared with that of streptomycin and isoniazid alone.

TABLE 7

THE CHEMOTHERAPEUTIC ACTION OF ISONIAZID AND STREPTOMYCIN SINGLY AND IN COMBINATION

(*M. tuberculosis*, Strain Vallée: In Mice)

DAILY DOSAGE*		NUMBER OF MICE	PER CENT SURVIVAL (42 DAYS)	AVERAGE SURVIVAL TIME (42 DAYS)
SM (mg.)	INH (mg.)			
0.50	0.10	10	90	42.0
0.25	0.10	10	100	42.0
0.50	0.05	10	100	42.0
0.25	0.05	10	30	33.0
0.50	0.00	10	80	40.2
0.25	0.00	10	30	33.6
0.00	0.10	10	100	42.0
0.00	0.05	10	80	40.8
0.00	0.00	10	0	26.5

* Single daily subcutaneous injection of solution containing streptomycin (SM) and isoniazid (INH). Dosage of streptomycin expressed in terms of the equivalent weight of pure streptomycin base; that of isoniazid in terms of total weight of drug.

As illustrated in table 7, the results obtained in these experiments provide no evidence of an antagonistic effect between streptomycin and isoniazid when high dosages of each are administered concomitantly in experimentally infected mice. In similar experiments in guinea pigs, antagonism likewise was not demonstrable when high dosages of streptomycin and isoniazid were used in combination (table 8). When the daily dosage of streptomycin was low, however, too low to protect mice against a rapidly progressing form of the disease, the simultaneous administration of minimally effective therapeutic dosages of isoniazid resulted in inadequate protection. Whereas 0.5 mg. of streptomycin in combination with 0.05 mg. of isoniazid gave as complete protection as either 0.5 mg. of streptomycin or 0.05 mg. of isoniazid alone, nevertheless a combination of 0.05 mg. of isoniazid, a minimally effective dose, and 0.25 mg. of streptomycin, a

[8] We are indebted to Dr. Oscar Auerbach of the Veterans Administration Hospital, East Orange, New Jersey, for the histopathologic examination of these sections.

sub-therapeutic amount, was no more effective than 0.25 mg. of streptomycin alone (table 7). Low daily dosages of streptomycylidene isonicotinyl hydrazine (0.25 mg. total weight), containing minimally effective therapeutic amounts of isoniazid (0.04 mg.) but sub-therapeutic amounts of streptomycin (0.18 mg.), in like manner, were no more effective than dosages of streptomycin comparable to the amounts of streptomycin present in the streptomycylidene isonicotinyl hydrazine (table 9).

TABLE 8

THE CHEMOTHERAPEUTIC ACTION OF STREPTOMYCIN AND ISONIAZID IN EXPERIMENTAL TUBERCULOSIS OF GUINEA PIGS

(Strain H37Rv)

DAILY DOSAGE (MG./KG.)*		NUMBER OF ANIMALS	PER CENT SURVIVAL (70 DAYS)	LUNG CULTURES FOR TUBERCLE BACILLI: NUMBER POSITIVE/NUMBER CULTURED (SACRIFICED 70 DAYS)
SM	INH			
40	0	10	100	3/10
20	0	10	100	2/10
10	0	10	100	4/10
0	40	10	100	0/10
0	20	10	100	1/10
0	10	10	100	0/10
0	5	9	100	1/9
0	2.5	10	100	0/10
40	10	9	100	0/9
20	5	10	100	0/10
10	2.5	9	100	0/9
0	0	29	97	20/29

* Subcutaneous therapy: 2 doses per day, one every twelve hours. Therapy started twenty-four hours after infection. Dosages of isoniazid (INH) expressed in terms of total weight of drug; dosages of streptomycin expressed in terms of equivalent weights of streptomycin base.

All of the chemotherapeutic studies reported above represent experiments in which treatment was initiated on the day of, or the day following, infection. All represent therefore a prophylactic type of experiment.

A single experiment was conducted in which initiation of therapy in a portion of the animals was delayed until the seventh day following infection. In an acute and rapidly progressing disease process, such as that produced by the Vallée strain (LD_{50} = 24 days), it was assumed that the infection was well advanced by the seventh day. Streptomycin and isoniazid were used alone and in combination in the treatment of such animals. As indicated in table 10, the chemotherapeutic action of these two agents, singly and in combination, was as great in those animals in which treatment was started one week after infection as in those in which therapy was started on the day of infection.

Persistence of M. tuberculosis in tissues: In the series of animals which received

immediate and continued therapy, a higher incidence of lung cultures positive for *M. tuberculosis* was obtained from those animals which survived under streptomycin therapy than from those which survived under isoniazid therapy. With all therapeutic regimens used, the incidence of cultures positive for tubercle bacilli from the lungs of animals surviving drug therapy was greater in those animals whose course of treatment was started on the seventh day than in those whose treatment was started on the first day. Guinea pig inoculation of lung and/or spleen homogenates from animals who survived under drug therapy

TABLE 9

THE CHEMOTHERAPEUTIC ACTION OF STREPTOMYCIN, ISONIAZID, AND STREPTOMYCYLIDENE ISONICOTINYL HYDRAZINE IN MICE

(*M. Tuberculosis*, Strain Vallée)

DAILY DOSAGE†		NUMBER OF MICE	PER CENT SURVIVAL (42 DAYS)	AVERAGE SURVIVAL TIME (42 DAYS)
Compound Used	SM (mg.)	INH (mg.)			
SIH	0.70*	0.16	20	100	42.0
	0.35*	0.08	20	75	39.7
	0.18*	0.04	20	30	34.4
	0.07*	0.016	16	19	28.6
SM	1.00	0.0	20	95	41.8
	0.50	0.0	20	85	39.8
	0.25	0.0	20	55	34.8
INH	0.0	0.25	20	95	41.0
	0.0	0.10	20	100	42.0
	0.0	0.05	20	90	41.4
	0.0	0.0	30	0	24.2

* Streptomycin (SM) and isoniazid (INH) administered concomitantly in the form of streptomycylidene isonicotinyl hydrazine in daily dosages equivalent to 1.0, 0.5, 0.25, and 0.10 mg., total weight, respectively; complete hydrolysis assumed.

† Dosages of isoniazid expressed in terms of total weight of drug; dosages of streptomycin expressed in terms of equivalent weights of pure streptomycin base.

indicated a higher percentage of animals harboring viable bacilli than was apparent by direct culture.

Based on the data obtained by both direct culture of lung tissues and guinea pig inoculation of lung and/or spleen homogenates, it is apparent that 100 per cent of drug-treated animals which survived delayed therapy with streptomycin, isoniazid, or streptomycylidene isonicotinyl hydrazine harbored viable tubercle bacilli at the time of autopsy. Furthermore, 100 per cent of those in which treatment with streptomycin alone was initiated on the day of infection appeared to harbor viable bacilli at the time of autopsy. Only 40 to 44 per cent of animals which received isoniazid alone or in combination in this manner, however, appeared to be harboring viable tubercle bacilli at the time of autopsy. Based on

this single experiment, therefore, neither isoniazid nor streptomycin, singly or in combination, was capable of eradicating the invading organisms completely

TABLE 10

THE CHEMOTHERAPEUTIC ACTION OF STREPTOMYCIN AND ISONIAZID SINGLY AND IN COMBINATION

Comparison of Delayed and Early Therapy
(Vallée Strain)

DOSAGE*								NUMBER OF MICE		
Mg. Daily		Number of Mice	PER CENT SURVIVAL*	AVERAGE SURVIVAL TIME* (DAYS)	LUNG CULTURES FOR TUBERCLE BACILLI: NUMBER POSITIVE/ NUMBER CULTURED	NUMBER INOCULATIONS TO GUINEA PIGS*		Producing in Guinea Pigs		With Viable Bacilli by Culture and/or Guinea Pig Inoculation
SM	INH					Mice	Organs	Local lesion	Progressive disease†	
Immediate Therapy										
3	0.75	10	90	47.7	1/9	9	14	3/9	0/9	4/9
2	0.50	10	80	44.6						
1	0.25	10	90	50.1						
3	—	10	100	51.0	10/10	10	15	9/10	10/10	10/10
2	—	10	100	51.0						
1	—	10	100	51.0						
—	0.75	10	100	51.0	1/10	10	15	4/10	4/10	4/10
—	0.50	10	100	51.0						
—	0.25	10	100	51.0						
Delayed Therapy										
3	0.75	10	100	58	5/10	10	15	6/10	7/10	10/10
2	0.50	10	80	52.3						
1	0.25	10	90	53.3						
3	—	10	(50)	47.6	10/10	5	8	10/10	10/10	10/10
2	—	10	100	58.0	10/10	5	7	2/10	10/10	10/10
1	—	10	100	58.0						
—	0.75	10	70	50.9	10/10	5	8	10/10	10/10	10/10
—	0.50	10	100	58.0	2/10	5	7	4/10	8/10	10/10
—	0.25	10	90	53.3						
Controls										
—	—	10	0	34.2						
Saline		20	5	29.4						

* Animals which received immediate therapy evaluated and sacrificed at fifty-one days after infection; animals which received delayed therapy evaluated and sacrificed at fifty-eight days after infection.

† Guinea pigs which did not die before the one hundred and sixty-seventh day were sacrificed on that day and observed for extent of disease.

although both caused a marked suppression of the disease even when therapy was started late.

Emergence of resistance in vivo: Although on high dosages, as reported pre-

viously, the incidence of lung cultures positive for tubercle bacilli in surviving isoniazid-treated animals was low; nevertheless, with smaller dosages, a high incidence of positive lung cultures was obtained from treated animals. A single experiment has been conducted to date to determine the incidence of resistance among such strains. Growth in the presence of 10 γ or more of streptomycin

TABLE 11

EMERGENCE OF DRUG-RESISTANT TUBERCLE BACILLI IN VIVO

(Vallée Strain of *M. tuberculosis*)

THERAPY			NUMBER OF MICE CULTURED*	SUSCEPTIBILITY: γ/ML.					
Agent Used	Mg. per Day			Streptomycin			Isoniazid		
	SM	INH		<5	5–10	>10	<2	2–4	>4
SM	1.00	—	12	3	7	2			
	0.50	—	9	0	6	3			
	0.25	—	8	0	6	2			
INH	—	0.25	12				11	0	1
	—	0.10	15				11	0	4
	—	0.05	12				6	0	6
	—	0.025	3				2	0	1
SIH†	0.70	0.16	11	5	2	2	8	0	3
	0.35	0.08	13	8	5	0	7	0	5
	0.175	0.04	10	6	2	2	6	0	2
	0.07	0.016	9	1	7	1	7	0	2
SM plus INH	0.5	0.025	3	1	1	0	2	0	1
	0.5	0.05	3	3	0	0	3	0	0
	0.5	0.10	5	0	4	0	3	0	2
	0.25	0.05	9	6	1	0	6	0	2
	0.25	0.10	8	6	1	0	5	0	3
Total			142	39	42	12	77	0	32
Per cent				42	45.1	12.9	70.6	0	29.4

* Lungs from mice cultured at time of death or when sacrificed at completion of experiment.

† Streptomycylidene isonicotinyl hydrazine: 70 per cent streptomycin, 16 per cent isoniazid, by weight; complete hydrolysis assumed.

was accepted as evidence of resistance to this antimicrobial agent, while minimal inhibitory concentrations ranging from 5 to 10 γ per ml. were accepted as an indication of partial resistance. With isoniazid, minimal inhibitory concentrations of 2 to 4 γ per ml. were accepted as evidence of partial resistance, while growth in the presence of 4 or more γ per ml. was accepted as evidence of resistance. On this basis, as illustrated in table 11, the incidence of resistance to isoniazid in this single experiment was 29.4 per cent, while the incidence of strains resistant to streptomycin was 12.9 per cent. No strains partially resistant

to isoniazid were recovered. In contrast, 45.1 per cent of those strains tested for susceptibility to streptomycin were partially resistant to this agent. It is of interest that, of 60 strains recovered from animals which had been treated either with streptomycylidene isonicotinyl hydrazine or with mixtures of streptomycin and isoniazid, 27 were susceptible to both streptomycin and isoniazid; 5 were susceptible to streptomycin but resistant to isoniazid; 13 were susceptible to

TABLE 12

EMERGENCE OF DRUG-RESISTANT TUBERCLE BACILLI IN VIVO

(Vallée Strain of *M. Tuberculosis*)

THERAPY			NUMBER OF STRAINS					
Agent Used	Mg. Daily		SM-S* INH-S	SM-S INH-R	SM-R INH-S	SM-PR INH-S	SM-PR INH-R	SM-R INH-R
	SM	INH						
SIH	0.70	0.16*	5	0	1	0	2	1
	0.35	0.08	6	1	0	1	4	0
	0.175	0.04	4	0	0	2	0	2
	0.07	0.016	0	1	0	7	0	1
Total			15	2	1	10	6	4
Per cent							26.3	
SM plus INH	0.50	0.10	0	0	0	2	2	0
	0.25	0.05	4	1	0	0	1	0
	0.50	0.05	3	0	0	0	0	0
	0.25	0.10	4	2	0	0	1	0
	0.50	0.025	1	0	0	0	1	0
Total			12	3	0	2	5	0
Per cent							22.7	

S = susceptible; PR = partially resistant; R = resistant; SIH = streptomycylidene isonicotinyl hydrazine.

* These values assume full hydrolysis of streptomycylidene isonicotinyl hydrazine.

isoniazid and resistant, or partially resistant, to streptomycin; and 15 were resistant to isoniazid and also either partially or fully resistant to streptomycin.

Strains partially or fully resistant to both streptomycin and isoniazid were recovered both from streptomycylidene isonicotinyl hydrazine-treated animals and from animals which had been treated with mechanical mixtures of streptomycin and isoniazid (table 12).

DISCUSSION

Early reports on isoniazid suggested that this compound possessed a remarkable degree of antituberculous activity. Indeed, in some laboratories its *in vitro* activity appeared to be extremely high in comparison with that of streptomycin.

In the experience of the present writers, as indicated by the data reported herein, the *in vitro* activity of isoniazid equals and at times exceeds by two- to threefold the activity of streptomycin. In like manner, as indicated also by the data herein, isoniazid is effective *in vivo* as a chemotherapeutic agent in the control of experimental tuberculous infections in less than one-third of the dosage (comparison by weight) than is required with streptomycin.

The greater activity of isoniazid *in vivo*, in contrast to that of streptomycin, may be a reflection of its greater ability to be transferred across the host cell membrane and, hence, to act upon intracellular organisms, as demonstrated by Suter (18) and by Mackaness and Smith (19). It may be a reflection, furthermore, of differences in the pharmacologic action of the two agents, in the concentrations which reach the site of the lesion, and in the length of time that they remain available at the site of the lesion. As shown herein, low concentrations of isoniazid may exert a more pronounced bacteriostatic effect than similar concentrations of streptomycin, even though the latter agent is the more bactericidal when present in higher concentrations. As pointed out by Eagle and associates (20), the number of viable bacteria in any clump or "unit" of tubercle bacilli may influence the degree of bacteriostasis or bactericidal action which may result in a study of this sort. Thus, the data presented herein are qualitative in nature and exact comparisons cannot be made with growth curve data presented by other investigators.

If the term *synergism* is used to designate the concept of two agents acting together to produce an effect greater than that which could be achieved by the same quantity of each agent alone, then it must be assumed from the data presented herein that streptomycin and isoniazid at times may act synergistically both *in vitro* and *in vivo*. This is evidenced by the increased bactericidal action of streptomycin in the presence of small amounts of isoniazid and by the greater degree of chemotherapeutic action when the two agents were used concomitantly. It is of interest that this synergistic effect could be observed *in vivo* only when proper dosages were used, and that minimally effective amounts of isoniazid in combination with subeffective amounts of streptomycin were no more effective than these subeffective amounts of streptomycin alone. Although this lack of effect is not indicative of a true antagonism between streptomycin and isoniazid, it perhaps lends support to the belief held by Jawetz and associates (21) to the effect that an excess of one or both antimicrobial agents is necessary for optimum effects when combined therapy is used.

That strains resistant to the action of streptomycin emerge rapidly from otherwise susceptible cultures is a well-known phenomenon. Evidence has been presented by many to suggest that strains resistant to isoniazid also appear at a rapid rate. Data based on the limited number of strains utilized in the present study have confirmed these observations with isoniazid and have indicated that resistance appears to develop through a two-stage process, as does streptomycin resistance.

From the data presented, it is apparent that streptomycin and isoniazid in combination in the form of streptomycylidene isonicotinyl hydrazine significantly delay but do not prevent the emergence of organisms resistant to these sub-

stances. Since streptomycin-resistant strains not previously in contact with isoniazid, and isoniazid-resistant strains not previously in contact with streptomycin, are susceptible to isoniazid and streptomycin, respectively, it is of interest that strains made resistant to streptomycylidene isonicotinyl hydrazine were fully resistant to streptomycin and also to isoniazid. Whether this is indicative of a double mutation brought about by small amounts of unhydrolyzed streptomycylidene isonicotinyl hydrazine or whether it indicates that emergence of resistance in this instance is brought about by some mechanism other than a purely genetic one cannot be stated from the information available. It is possible that streptomycin and isoniazid may act upon the cell at different sites or that isoniazid may interfere with some mechanism of importance in relation to drug resistance.

In a small series of experiments reported herein, strains resistant to the action of isoniazid and/or streptomycin were recovered frequently from isoniazid- or streptomycin-treated animals. The *in vivo* emergence of strains partially or fully resistant to both streptomycin and isoniazid from animals treated with combined therapy confirms the *in vitro* observations that cross-resistance may occur. The fact that a portion of these resistant strains was recovered from animals receiving a mechanical mixture of streptomycin and isoniazid seems to eliminate the possibility that cross-resistance may have occurred through a double mutation brought about by traces of unhydrolyzed streptomycylidene isonicotinyl hydrazine.

Insufficient data are available to permit the assumption that the impressions gained from the observations reported herein and discussed above would apply to all strains of *M. tuberculosis*. Indeed, there is some evidence to suggest that the action of isoniazid alone and in combination with streptomycin against *M. butyricum* may differ from its action against the H37Rv strain of *M. tuberculosis*. Further evidence is needed to confirm this impression and to prove whether or not all strains of *M. tuberculosis* var. *hominis* parallel the strains used in the present study.

SUMMARY

Observations *in vitro* and *in vivo* are presented which indicate the high degree of antituberculous activity of isoniazid against both streptomycin-susceptible and streptomycin-resistant organisms. It was found that (*a*) the emergence of resistance to combinations of streptomycin and isoniazid occurs more slowly than to either compound alone; (*b*) neither compound is capable of preventing the ultimate emergence of resistance to the other; (*c*) cross-resistance between streptomycin and isoniazid may occur under certain conditions despite the fact that isoniazid is effective against streptomycin-resistant organisms not previously in contact with isoniazid, and streptomycin is effective against isoniazid-resistant organisms not previously in contact with streptomycin.

It was concluded, therefore, that streptomycin and isoniazid, when used under appropriate conditions and in adequate quantities, may act in a synergistic manner both *in vitro* and *in vivo*.

SUMARIO

Actividad in Vitro e in Vivo de la Estreptomicina y la Isoniacida por sí Solas y Combinadas

Preséntanse observaciones *in vitro* e *in vivo* que reflejan la elevada actividad antituberculosa desplegada por la isoniacida contra los microbios tanto estreptomicinosusceptibles como estreptomicinorresistentes. Observose que: (*a*) la formación de resistencia a las combinaciones de estreptomicina e isoniacida aparece más lentamente que a uno u otro compuesto por sí solo; (*b*) ni uno ni otro compuesto es capaz de impedir la formación a la larga de resistencia al otro; (*c*) puede aparecer en ciertas circunstancias resistencia cruzada entre la estreptomicina y la isoniacida, a pesar de ser la isoniacida eficaz contra gérmenes estreptomicinorresistentes que no han estado antes en contacto con la isoniacida y de ser la estreptomicina eficaz contra gérmenes isoniacidorresistentes que no han estado en contacto antes con la estreptomicina. Dedúcese, pues, que usadas en condiciones apropiadas y a dosis adecuadas, la estreptomicina y la isoniacida pueden obrar sinérgicamente tanto *in vitro* cuanto *in vivo*.

RESUME

Activité in vitro et in vivo de la streptomycine et de l'isoniazide, séparément ou en association

Présentation d'observations *in vitro* et *in vivo* indiquant le haut degré d'activité antituberculeuse de l'isoniazide contre les germes streptomycino-sensibles et streptomycino-résistants. On a trouvé que: a) l'apparition de la résistance à la combinaison streptomycine-isoniazide se manifeste plus lentement que lorsque l'un ou l'autre de ces composés est administré séparément, b) aucun de ces composés n'est capable de prévenir l'apparition ultérieure de la résistance à l'autre, c) la résistance croisée ("cross-resistance") entre la streptomycine et l'isoniazide peut se produire dans certaines conditions, en dépit de l'efficacité de l'isoniazide contre des germes streptomycino-résistants n'ayant pas été antérieurement en contact avec la streptomycine. En conséquence, les auteurs concluent que la streptomycine et l'isoniazide, utilisées dans des conditions appropriées et en quantité adéquate, peuvent avoir une action synergique à la fois *in vitro* et *in vivo*.

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A TOXIC LIPID COMPONENT OF THE TUBERCLE BACILLUS ("CORD FACTOR")[1]

II. Occurrence in Chloroform Extracts of Young and Older Bacterial Cultures

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INTRODUCTION

In the preceding paper in this series (1), the fractionation of "cord factor" derived from tubercle bacilli and the purification of the toxic component of the factor were described. It was shown that the latter represents but a small fraction of the total extract and that the yield of this material is poor. As the work progressed, the need for better sources of active material became more urgent, and methods were developed to improve the rendering of extraction. The present paper describes such various procedures.

"Cord factor" had first been obtained by superficial extraction of young cultures of virulent tubercle bacilli (2). This method furnished a crude product which was adequate for certain animal experiments which required only small amounts of material (2, 3), but it became unsatisfactory when larger quantities had to be at hand for chemical analysis of the extracts. Bacterial cultures older than approximately two weeks which had a more luxuriant growth gave greater yields upon extraction with petroleum ether, but the relative toxicity of the extracts dropped as the cultures grew older and larger doses had to be injected to produce the same effects. Since the toxic component was of particular interest, this procedure was obviously no solution to the problem. Equally unsuccessful were attempts to improve the yield by extracting the bacilli more completely with petroleum ether in a Soxhlet apparatus. While more material was obtained by this method, the proportion of the toxic principle did not increase correspondingly. The previous observation was confirmed that the active material which appeared to be located at the surface of the bacterial cell was easily obtainable by superficial petroleum ether extraction of short duration, but that any attempt at improving the yield would have to be made through different and more thorough methods of extraction.

It had been found that treatment with petroleum ether was not very injurious to the bacillus. The organisms were able to multiply *in vivo* as well as *in vitro* after they had been suspended in petroleum ether for long periods of time (2).

The first question that arose was whether the tubercle bacillus still contained toxic material after extraction with petroleum ether. Previous observations suggested that the material removed by this solvent is a secretory product released by the bacillus into the surrounding medium, provided the organisms are suspended in a lipophilic environment where "cord factor" is sufficiently soluble. Indeed, it was possible to recover material with "cord factor" toxicity from

[1] From the Division of Applied Immunology, The Public Health Research Institute of The City of New York, Inc., New York 9, New York.

culture filtrates of media with an unusually high concentration of Tween 80,[2] in which "cord factor" seems to be soluble (3).

Despite the indication of this bacterial constituent being a secretory product which is either discharged into the medium or accumulated at or near the surface of the cells, depending on the prevailing physicochemical conditions, it seemed worth searching for a component with "cord factor" toxicity in bacterial extracts obtained with classical methods of extraction. The fact that no such constituent had previously been found by other workers was not too surprising. The relative amounts are likely to be extremely small and the physical properties of the material are grossly similar to those of other constituents present in much higher proportions. Moreover, the specific material has to be traced with the help of a sensitive test, a procedure which is not followed by most chemical workers in this field.

The attempts to isolate "cord factor" from sources other than petroleum ether extracts of young bacterial cultures appeared more promising after comparable toxic properties had been found in preparations of Lederer, Asselineau, and their collaborators. In an effort to clear up possible inconsistencies, during the past two years information has been exchanged with the French group and approximately seventy of their isolation products have been biologically tested. Some fractions, chiefly the waxes C and D, had the characteristic toxicity for mice (4). This suggested that they contained various amounts of material similar to the toxic component of "cord factor," which made it promising to search for toxic material in organisms extracted according to Anderson's methods.

The present paper describes the isolation and chemical properties of a toxic component similar or identical to the material present in petroleum ether extracts of young bacterial cultures. This component was obtained from young as well as from old cultures with the methods of Anderson.

EXPERIMENTAL

Bacteria: The bacilli were grown as surface cultures on a modified Lockemann medium (5) or on Long's synthetic medium and harvested as indicated below.

Biological assay of bacterial extracts: A detailed description of the method used for the quantitative determination of the toxic properties of an extract has previously been published (3). This paper should be consulted for all particulars of the procedure.

Isolation of the "purified wax": Three different types of bacterial cultures were used: (*1*) two-week-old cultures of live human-type tubercle bacilli of the H37Rv strain; (*2*) similar cultures which have previously been extracted with petroleum ether; (*3*) heat-killed, dried twelve-week-old, pooled cultures of the PN, DT, and C strains.[3]

All cultures were extracted in the same way by immersion for several days in a mixture of equal parts of ethanol and ether in the state in which they were originally obtained, that is:

Bacilli (No. 1), after filtering from the culture medium and washing on a Buchner funnel

[2] Tween 80 is the proprietary name for the polyoxyethylene derivative of Sorbitan monooleate, obtainable from the Atlas Powder Co., Wilmington, Delaware.

[3] The authors wish to thank Dr. H. D. Piersma of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, for the generous supply of these bacterial cultures.

Bacilli (No. 2), after petroleum ether extraction and filtering on a Buchner funnel
Bacilli (No. 3), in the form of dried bacterial masses

During this extraction the solvent turned deep yellow. It was decanted off, discarded, and the bacilli were transferred to a Buchner funnel, where the extraction was continued by passing fresh solvent through the bacterial residue until the filtrate was colorless and practically free of bacterial lipids. The ether-ethanol was then replaced by chloroform and the extraction continued in the same manner. The contact with chloroform turned the bacteria into a sticky, gluelike mass which clogged the filter paper and rendered the filtration difficult. This adverse effect could be minimized by drying the bacteria on a suction filter before the extraction with chloroform. No such glue formation was observed with the bacilli (No. 3) which were completely dry before extraction.

Upon extraction with chloroform, the filtrate turned yellow again, becoming colorless toward the end of the procedure. The chloroform extracts were then passed through a Seitz filter, the solvent was evaporated, and the remaining oily residue dissolved in ether. Addition of three parts of methanol resulted in precipitation of the "purified wax" (PW) (6). The yellow supernatant containing the "soft wax" (6) was separated by filtration and the precipitate washed with methanol. After drying, it formed a yellowish powder.

Separation of the "Purified Wax" into a Low Melting and a High Melting Fraction

Separation with acetone: Asselineau has shown (7) that PW could be further fractionated by extraction with boiling acetone. In his experiments, which were confirmed by the present writers, the acetone extracts yielded, upon cooling, a white amorphous low melting fraction (C), melting point 40° to 55°C., while the insoluble residue represented a high melting fraction (D), melting point 160° to 240°C.[4]

It was found that both fractions obtained by this method contained toxic material but that its distribution between the two fractions was dependent on the duration of the extraction procedure. Thus, refluxing of PW for several days with frequently renewed portions of boiling acetone still yielded small amounts of acetone-soluble material. After prolonged treatment, however, the toxic material was mostly concentrated in the acetone-soluble fraction C. The latter can again be separated into a hot acetone-soluble and a boiling acetone-soluble part which results in the following three fractions:

1. C_1, soluble in hot acetone below the boiling point
2. C_2, soluble only in boiling acetone
3. D, insoluble in boiling acetone

The distribution of the toxic properties among the three fractions may be seen in table 1.

Toxic material was found in extracts from two-, six-, and twelve-week-old cultures. Its effects on mice could not be distinguished biologically from the toxicity exerted by petroleum ether extracts of young bacterial cultures. Younger cultures, however, yielded slightly more PW. Previous extraction of the bacilli with petroleum ether seemed to reduce the yield of this material (table 2).

Separation with ether-methanol:[5] The higher melting fraction D was found to be less soluble in ether than the low melting fraction C. When methanol was slowly added, while stirring, to a concentrated solution of PW in ether, a sticky precipitate was formed after the addition of approximately one part methanol. The clear supernatant was decanted off and more methanol added to it. This resulted in the formation of a voluminous white precipitate, melting point 47° to 52°C. (fraction C). The first precipitate (D), melting point 130° to 150°C., was still impure as shown by the broad melting point and the content of toxic material. Repeated treatment of fraction D with ether-methanol yielded more material of the low melting fraction and resulted in a further purification of D. A purified

[4] The designations C and D are used according to Asselineau and Lederer to distinguish the lower from the higher melting fractions of "purified wax." The higher melting fraction D is a lipopolysaccharide (7).

[5] All experiments referred to from here on were carried out with PW isolated from pooled twelve-week-old cultures of the PN, DT, and C strains grown and harvested as described above.

sample of fraction D (melting point 185° to 187°C.) was obtained in this manner from PW of twelve-week-old cultures. It was nontoxic.

This method for obtaining the toxic fraction C with which the present study was concerned proved to be more efficient than the fractionation with boiling acetone, as shown by a typical example summarized in table 3.

TABLE 1

THE DISTRIBUTION OF THE TOXIC PRINCIPLE AMONG VARIOUS FRACTIONS OF "PURIFIED WAX" OBTAINED FROM BACTERIAL CULTURES OF DIFFERENT AGE

BACILLI	FRACTION	MELTING POINT	TOXICITY*
2 weeks	C_1	50° to 59°C.	0
2 weeks	C_2	40° to 49°C.	+
2 weeks	D	85° to 90°C.	0
6 weeks	$C_1 + C_2$	38° to 45°C.	+
6 weeks	D	150° to 160°C.	0
12 weeks	C_1	50° to 55°C.	0
12 weeks	C_2	42° to 53°C.	+
12 weeks	D	130° to 140°C.	0

* + indicates the preparation was toxic; 0 indicates it was nontoxic for mice.

TABLE 2

THE YIELD OF "PURIFIED WAX" FROM BACTERIAL CULTURES OF DIFFERENT AGE

BACTERIAL CULTURES	WEIGHT PER CENT OF BACTERIAL DRY WEIGHT		
	Total "Purified Wax" (PW)	Acetone-Soluble (Toxic) (C_1 C_2)	Acetone-Insoluble (D)
H37Rv 2 weeks	3.2	1.2	2.0
H37Rv 2 weeks*	1.9	0.8	1.1
H37Rv 6 weeks	0.7	0.2	0.5
PN, DT, C 12 weeks	2.6	0.4	2.2

* Previously extracted with petroleum ether.

TABLE 3

EFFICACY OF VARIOUS METHODS OF EXTRACTION IN ISOLATING FRACTION C OF "PURIFIED WAX"

TREATMENT	RECOVERY OF FRACTION C IN PER CENT OF TOTAL PW
Extraction with boiling acetone for 15 minutes	1 per cent
Refluxing for 5 days	15 per cent
Ether-methanol fractionation	40 per cent

Purification by Chromatography

The starting material for chromatographic analysis was fraction C of the "purified wax" obtained from the pooled twelve-week-old dry bacilli by ether-methanol precipitation as described in the foregoing section.

Fractionation on magnesium silicate-Celite: Since it was found by Bloch, Sorkin, and Erlenmeyer (1) that toxic material can be efficiently concentrated by chromatography on magnesium silicate-Celite 545,[6] this method was used for further purification. However,

[6] We are indebted to the Johns-Manville Corporation, New York, New York, for a gift of Celite 545.

the adsorbent was heated at atmospheric pressure and not *in vacuo*, which appeared to result in a milder adsorbent of lesser activity. The yield of the eluted material was determined by weight after evaporating the solvents. The material was applied to the column in petroleum ether solution and eluted with petroleum ether and mixtures of petroleum ether-benzene, benzene-ether, and ether-methanol successively. Preliminary studies showed that the active material was strongly adsorbed on the column and eluted only in the tail fractions with ether containing 5 to 10 per cent of methanol.[7] The active fractions amounted to approximately 10 per cent of the starting material. Since it was all eluted with ether-methanol, a simplified chromatographic procedure was adopted which eliminated approximately 80 per cent of the nontoxic fractions without collecting numerous individual samples. An example is given in table 4.

TABLE 4

CHROMATOGRAM I

SEPARATION OF THE TOXIC PRINCIPLE BY RAPID CHROMATOGRAPHY ON MAGNESIUM SILICATE-CELITE

Starting Material: 2.15 Gm. "Purified Wax," Fraction C

FRACTION	ELUTED WITH 100 ML. OF:	MG.	TOXICITY*
1	petrol ether	883.7	0
2	petrol ether	32.8	
3	petrol ether-benzene 1:1	70.6	0
4	benzene	184.8	
5	benzene-ether 1:1	110.5	0
6	ether	103.7	
7	ether plus 25% methanol	424.7	+
8	ether plus 25% methanol	56.3	
Recovered		1,867.1 = 87%	

* Symbols as in table 1.

After this preliminary step, the active material from the ether-methanol eluate was further purified by several successive chromatographies until a separation into active and inactive fractions was no longer possible. The following scheme illustrates this process of purification:

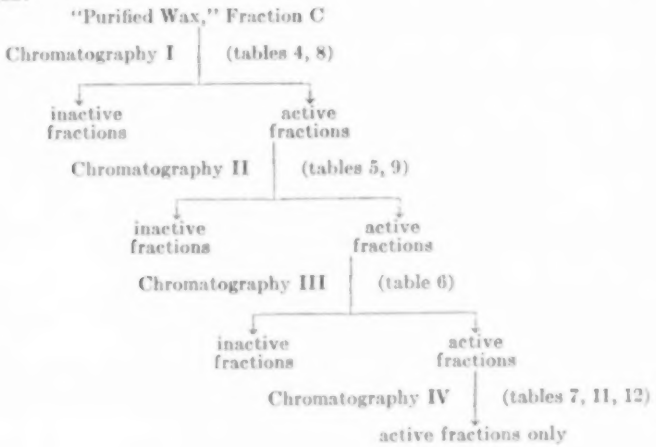

TABLE 5

CHROMATOGRAM II

CHROMATOGRAPHY OF 1.6253 GM. OF ETHER-METHANOL ELUATES FROM CHROMATOGRAMS AS THE ONE SHOWN IN TABLE 1 DISSOLVED IN 80 ML. OF PETROL ETHER AND ADSORBED ON 50 GM. OF MAGNESIUM SILICATE-CELITE

FRACTION	ELUTED WITH	MG.	TOXICITY TEST			
			AMOUNT INJECTED PER DOSE	Number of Injections	Number of Mice Dying with Typical Symptoms	Degree of Toxicity*
			mg.			
1	200 ml. petrol ether	traces				
2	10 ml. petrol ether-benzene 1:1	1.3				
3	10 ml. petrol ether-benzene 1:1	0.6				
4	10 ml. petrol ether-benzene 1:1	0.5				
5	10 ml. petrol ether-benzene 1:1	0.5				
6	30 ml. petrol ether-benzene 1:1	1.0				
7	30 ml. petrol ether-benzene 1:1	0.3				
8	10 ml. benzene-ether 1:1	11.5	0.1	2	0 of 5	0
9	10 ml. benzene-ether 1:1	22.5				
10	10 ml. benzene-ether 1:1	69.7	0.1	3	1 of 5	+
11	10 ml. benzene-ether 1:1	110.5				
12	10 ml. benzene-ether 1:1	109.2	0.1	2	0 of 5	0
13	10 ml. benzene-ether 1:1	71.4				
14	10 ml. benzene-ether 1:1	47.1	0.1	2	0 of 5	0
15	10 ml. benzene-ether 1:1	37.3				
16	10 ml. benzene-ether 1:1	24.9				
17	10 ml. benzene-ether 1:1	19.1				
18	10 ml. benzene-ether 1:1	12.9				
19	10 ml. benzene-ether 1:1	12.6				
20	10 ml. benzene-ether 1:1	12.2				
21	10 ml. benzene-ether 1:1	13.4				
22	10 ml. ether	18.3	0.1	5	0 of 5	0
23	10 ml. ether	13.4				
24	10 ml. ether plus 1% methanol	15.3	0.1	5	0 of 5	0
25	10 ml. ether plus 1% methanol	11.1				
26	20 ml. ether plus 1% methanol	14.2	0.1	2	0 of 4	0
27	33 ml. ether plus 2% methanol	75.3	0.1	2	0 of 5	0
28	27 ml. ether plus 3% methanol	230.6	0.1	5	2 of 5	+
29	10 ml. ether plus 3% methanol	93.4	0.1	2	4 of 5	++
30	28 ml. ether plus 5% methanol	128.8	0.1	3	5 of 5	+++
31	10 ml. ether plus 5% methanol	52.1	0.1	2	4 of 5	++
32	30 ml. ether plus 8% methanol	75.2	0.1	4	3 of 5	++
33	30 ml. ether plus 8% methanol	41.0	0.1	4	4 of 5	++
34	50 ml. ether plus 15% methanol	24.8	0.1	3	4 of 5	++
35	50 ml. ether plus 15% methanol	8.1	0.1			
Recovered		1,380.1 = 85%				

* For easier reading, the degree of toxicity is rated in an arbitrary scale ranging from 0 to +++.

Typical examples of successive chromatographic steps are shown in tables 5, 6, and 7. It can be seen that the active material was always eluted toward the end with ether containing 5 to 10 per cent methanol.[7] The toxic fractions from the second chromatography were pooled and rechromatographed (table 6). Two distinct peaks were obtained in this operation: a first peak, eluted with benzene-ether 1:1 with the nontoxic fractions; and a second peak containing the toxic components. In this instance, two injections of 10 γ were lethal for mice. Rechromatography of the active fractions of the second peak did not result in a further resolution (table 7).

TABLE 6

CHROMATOGRAM III

CHROMATOGRAPHY OF 269 MG. OF FRACTIONS 30 THROUGH 35 FROM CHROMATOGRAM II DISSOLVED IN 20 ML. OF PETROLEUM ETHER ADSORBED ON 10 GM. OF MAGNESIUM SILICATE-CELITE

FRACTION	ELUTED WITH:	MG.	TOXICITY TEST			
			Amount Injected per Dose	Number of Injections	Number of Mice Dying with Typical Symptoms	Degree of Toxicity*
			mg.			
1	10 ml. benzene-ether 1:1	22.3				
2	10 ml. benzene-ether 1:1	41.8	0.1	2	0 of 4	0
3	10 ml. benzene-ether 1:1	21.8				
4	10 ml. benzene-ether 1:1	11.7				
5	10 ml. benzene-ether 1:1	6.3				
6	10 ml. benzene-ether 1:1	5.2				
7	10 ml. benzene-ether 1:1	2.3				
8	10 ml. benzene-ether 1:1	1.6				
9	10 ml. ether plus 5% methanol	11.7				
10	10 ml. ether plus 5% methanol	78.4	0.1	2	2 of 4	+++
			0.01	2	3 of 4	
			0.007	2	0 of 4	
11	10 ml. ether plus 5% methanol	24.3				
12	10 ml. ether plus 5% methanol	8.9				
13	10 ml. ether plus 5% methanol	2.1				
14	10 ml. ether plus 5% methanol	1.2				
Recovered		239.6 = 89%				

* See footnote to table 5.

If columns of greater capacity were used in connection with an automatic fraction collector, the degree of purification which had previously been reached after three successive chromatographies was now achieved after two steps. Typical examples of two successive chromatographies are shown in tables 8 and 9. It was thus possible to separate the active material from the inactive components of the original mixture and to associate it with a chromatographic peak of apparent homogeneity and uniform toxicity. It was not possible any more, with this method of chromatography, to further separate the active material into toxic and nontoxic peaks. As will be shown in a later section, however, the

[7] When concentrated toxic material is rechromatographed, toxicity may sometimes be found in the fractions eluted with benzene-ether.

peak containing the toxic material was not a pure substance, and a different method of fractionation was sought.[8]

Properties of the Isolated Fractions

Throughout these experiments, the toxicity of isolated fractions was used as a tool for measuring the progress of purification achieved by chromatography. This method led to the separation of fractions with various degrees of toxicity from compounds which were completely inactive, and the bioassay proved to be

TABLE 7

CHROMATOGRAM IV

CHROMATOGRAPHY OF 48.2 MG. OF FRACTIONS 9 AND 11 THROUGH 14 FROM CHROMATOGRAM III DISSOLVED IN 10 ML. OF PETROLEUM ETHER ADSORBED ON 5 GM. OF MAGNESIUM SILICATE-CELITE

FRACTION	ELUTED WITH:	MG.	TOXICITY TEST			
			Amount Injected per Dose	Number of Injections	Number of Mice Dying with Typical Symptoms	Degree of Toxicity*
			mg.			
1	20 ml. petrol ether	0				
2–14	3 ml. benzene-ether 1:1 each	traces				
15–17	10 ml. benzene-ether 1:1 each	traces				
18	5 ml. ether plus 5% methanol each	3.2				
19	5 ml. ether plus 5% methanol each	41.2	0.1	2	4 of 4	+++
			0.01	1	4 of 4	
			0.007	2	1 of 4	
20	5 ml. ether plus 5% methanol each	4.7				
21	5 ml. ether plus 5% methanol each	1.4				
22–25	5 ml. ether plus 5% methanol each	traces				
Recovered		50.5 = 100%				

* See footnote to table 5.

a useful method of controlling the efficacy of the chromatographic purification. On the other hand, it was found that some of the physical properties of the fractions which could be quantitatively estimated were sufficiently characteristic to allow a differentiation of the individual components. Melting points, acidity, and infrared spectrum gave the most useful information for the distinction of the toxic fractions. The melting points were used for a more qualitative characterization of the purified fractions. The most toxic fractions had consistently lower melting

[8] While this manuscript was in preparation, it was found that chromatography on silica gel was a more efficient means of separation which yielded toxic fractions of higher purity. The sustaining data will be published in a forthcoming paper.

points (range, 40° to 48°C.) than the nontoxic acidic fractions (range, 52° to 57°C.).

The acidity was determined by microtitrations with a Gilmont ultra microburet which permitted readings of 0.5 mm³. The titrations were made in a nonaqueous system using a slight modification of the method described by Fritz and Lisicky (8). Samples of approximately 5 mg. were weighed, dissolved in 5 ml. of chloroform, and titrated with tenth-normal solution of sodium methylate prepared according to Fritz and Lisicky. Phenolphthalein as indicator gave sharp

TABLE 8

CHROMATOGRAM V

CHROMATOGRAPHY OF 5.0 GM. OF "PURIFIED WAX" FRACTION C DISSOLVED IN 100 ML. OF PETROLEUM ETHER ADSORBED ON 100 GM. OF MAGNESIUM SILICATE-CELITE

FRACTION	ELUTED WITH:	MG.
1	50 ml. petrol ether-benzene 1:1	845.7
2	50 ml. petrol ether-benzene 1:1	1,668.1
3	50 ml. petrol ether-benzene 1:1	1,138.1
4	50 ml. petrol ether-benzene 1:1	189.5
5	50 ml. benzene-ether 1:1	259.8
6	50 ml. benzene-ether 1:1	196.2
7	50 ml. benzene-ether 1:1	59.5
8	50 ml. benzene-ether 1:1	33.1
9	50 ml. ether plus 3% methanol	38.2
10	50 ml. ether plus 3% methanol	62.8
11	50 ml. ether plus 3% methanol	43.6
12	50 ml. ether plus 3% methanol	28.3
13	50 ml. ether plus 6% methanol	31.5
14	50 ml. ether plus 6% methanol	13.7
15	50 ml. ether plus 10% methanol	11.7
16	50 ml. ether plus 10% methanol	8.8
17	50 ml. ether plus 20% methanol	17.0
Recovered		4,645.6 = 92%

end points within 1 mm³. With chloroform alone, the blank was less than 1 mm³. Before each set of titrations, the titer of the base was determined by titrating against a standard solution of benzoic acid in chloroform. The values obtained differed not more than 3 per cent from each other. The reproducibility of the titrations of the lipid samples was within the accuracy of the analytical balance corresponding to approximately plus-minus 4 per cent for 5 mg. For 5 mg. samples of very low acidity corresponding to less than 4 mm.³ per mg., the deviations may be greater than plus-minus 5 per cent. The titrations have to be carried out in a room with low atmospheric humidity.

Further information concerning the chemical properties and the degree of purity of different fractions was obtained from infrared spectra.[9] The instrument

[9] The authors are greatly indebted to Dr. R. C. Gore, of the American Cyanamid Company, Stamford, Conn., and to all the members of this staff for the courtesy of running a

used was a Perkin-Elmer double-beam infrared recording spectrophotometer (model 21). Samples were prepared by gentle heating of 1 to 3 mg. substance between two rocksalt plates until they melted. The plates were then pressed to-

TABLE 9

CHROMATOGRAM VI

CHROMATOGRAPHY OF 245 MG. OF FRACTIONS 9 THROUGH 17 FROM CHROMATOGRAM V DISSOLVED IN 20 ML. OF PETROLEUM ETHER ADSORBED ON 12 GM. OF MAGNESIUM SILICATE-CELITE

FRACTION	ELUTED WITH:	MG.	TOXICITY TEST			
			Amount Injected per Dose	Number of Injections	Number of Mice Dying with Typical Symptoms	Degree of Toxicity*
			mg.			
1	10 ml. petrol ether	0.9				
2-3	10 ml. petrol ether	0				
4	10 ml. ether-benzene 1:1	23.0				
5	10 ml. ether-benzene 1:1	29.7	0.01	3	0 of 5	0
6	10 ml. ether-benzene 1:1	14.1				
7	10 ml. ether-benzene 1:1	6.8				
8	10 ml. ether-benzene 1:1	3.4				
9	10 ml. ether-benzene 1:1	2.3				
10	10 ml. ether-benzene 1:1	1.4				
11	10 ml. ether-benzene 1:1	0.3				
12	10 ml. ether-benzene 1:1	0.5				
13	10 ml. ether-benzene 1:1	0				
14	10 ml. ether plus 5% methanol	0				
15	10 ml. ether plus 5% methanol	0				
16	10 ml. ether plus 5% methanol	58.2	0.01	3	5 of 5	+++
17	10 ml. ether plus 5% methanol	15.4				
18	10 ml. ether plus 5% methanol	6.7				
19	10 ml. ether plus 5% methanol	2.1				
20	10 ml. ether plus 5% methanol	0.6				
21	10 ml. ether plus 5% methanol	0.7				
22	10 ml. ether plus 5% methanol	0.3				
23	10 ml. ether plus 5% methanol	0				
24	10 ml. ether plus 5% methanol	0.4				
25	10 ml. ether plus 5% methanol	0.9				
26	10 ml. ether plus 5% methanol	1.0				
27	10 ml. ether plus 5% methanol	1.0				
28	250 ml. ether plus 20% methanol	25.8				
Recovered		195.5 = 80%				

* See footnote to table 5.

gether so that the sample formed a thin transparent film, the thickness of which was adjusted with aluminum foils to approximately 0.03 mm.

great number of infrared spectra and for their invaluable help and advice in interpreting the results.

Three main components could be distinguished in the material eluted from the columns:

1. Fractions eluted in the first chromatography with petroleum ether and petroleum ether-benzene. The material precipitated from ether was a white powder, melting point 28° to 33°C., neutral. The infrared spectrum showed strong absorption at 1,731 cm.$^{-1}$, which is typical for esters (figure 9). All data agreed with those reported for the ester of phthiocerol with mycocerosic acid (7, 9, 10). This component constituted 70 to 85 per cent of fraction C of the "purified wax."

2. Fractions eluted with benzene-ether. After precipitation from ether, the material appeared as a white powder, melting point 51° to 55°C. The acidity corresponded to an equivalent weight of approximately 1,300. It constituted probably a mixture of acids of the mycolic acid type. It amounted to approximately 15 to 25 per cent of fraction C of the "purified wax."

3. Toxic fractions eluted with ether plus 5 per cent methanol.[7] This fraction was a white powder, melting point 42° to 47°C., with only traces of acidity; it represented about 1 to 3 per cent of fraction C of the "purified wax." Analytical data are presented below.

The present study is chiefly concerned with the toxic fractions. It was relatively easy to separate the nontoxic, low melting, neutral fractions from the remaining material, but the separation of the nontoxic acidic fraction (melting point 51° to 57°C.) from the toxic material (melting point 42° to 47°C.) was difficult. Both components were successfully obtained in relatively pure form, but the purest fractions of each were still contaminated with small proportions of the other.

A number of toxic fractions resulting from the different chromatographies were investigated. Some of the findings are summarized in table 10, but the following three typical examples are described in greater detail: a nontoxic product of high acidity; a fraction of higher toxicity with a lower degree of acidity; and the most toxic fraction obtained so far, which has a very low degree of acidity.

These three fractions were obtained from two new chromatographies (tables 11 and 12) which had been started from pools of the toxic and the nontoxic peaks, respectively, of the last steps of chromatographic purification (active and inactive peaks of tables 6, 7, and 9). These pools were made in order to obtain larger quantities of homogeneous, purified toxic material.

Example 1: Nontoxic fraction of high acidity (fraction 8, table 11):

Melting point: 53° to 55°C.

Acidity: 6.8 mm.^{3} 0.100 N sodium methylate per mg.

Infrared spectrum: The spectrum is shown in figure 1. It showed absorptions at 3,500, 3,050, 2,910, 2,850, 2,650, 1,705, 1,680, 1,470, 1,380, 1,300, 1,260, 1,205, 1,130, 1,100, 1,017, 962, 800, and 720 cm^{-1}. The strong bands at 2,910, 2,850, 1,470, and 720 are typical of CH_2, indicating the paraffinic character of the material. The CH_3 band at 1,380 was very strong, which suggests the presence of several branched chains. The sharp band between 3,500 and 3,520 is characteristic of unbonded —OH. Of particular interest is the C = O absorption between 1,680 and 1,725 cm^{-1}. It seemed to be an asymmetrical triplet with a weak maximum at 1,725, an intermediate at

TABLE 10

Correlation Between Infrared Spectra and Toxicity of Various Fractions

Fraction (Table and number) or designation	Spectrum figure	Estimated intensities of characteristic absorption bands†							
		Wave length cm.$^{-1}$:							
		3,500	3,350	3,050	1,725–1,710‡	1,680	1,380	1,100–1,000	Toxicity
		Correlations:							
		alcoholic OH		C=CH	C=O	acidic C=O	CH_2	C–O	
		unbonded	bonded						
Mycolic acid	4	++	0	0	0	+++	+++	0	0
Table 11, No. 8	1	+++	0	±	0	+++	+++	0	0
*	*	0	+	++	+	++	+	+	±
Table 11, No. 23	2	0	+	+	+	++	++	+	+
Table 11, Nos. 24 and 25	*	0	++	++	++	+	+	++	++
*	*	0	++	++	++	+	+	+++	++–+++
Table 12, starting material	8	0	++	+	++	+	+	+++	++–+++
Table 12, Nos. 27–30	*	0	+++	++	++	+	+	+++	++–+++
*	*	0	+++	++	+++	+	+	+++	++–+++
*	*	0	+++	++	+++	+	+	+++	++–+++
Table 12, No. 18	3	0	+++	++	+++	±	+	+++	+++
PP	6	0	+++	++	+++	±	+	+++	+++
PP, reduced	5	0	+++	++	0	0	+	+++	0
Table 12, starting material saponified	7	+	±	±	+	+++	+	++	0

* means that the chromatography from which this fraction is derived is not listed in this paper.

† Absorption intensities and the toxicity are listed in an arbitrary scale from 0 to +++.

‡ Since acidic and neutral C = 0 absorption are overlapping in this region, an estimate of the absolute intensity would be of little significance. The estimation of the intensity of the neutral C = 0 absorption, therefore, is based on a comparison of the C = 0 absorption at 1,725–1,710 cm.$^{-1}$ with the intensity of the acidic C = 0 absorption at 1,680 cm.$^{-1}$ and rated as follows:

0 absorption intensity at 1,725–1,710 considerably less than absorption at 1,680 cm.$^{-1}$

+ absorption intensity at 1,725–1,710 approximately equal to absorption at 1,680 cm.$^{-1}$

++ absorption intensity at 1,725–1,710 stronger than absorption at 1,680 cm.$^{-1}$

+++ absorption intensity at 1,725–1,710 much stronger than absorption at 1,680 cm.$^{-1}$

1,705, and a very strong, sharp peak at 1,680. This characteristic peak was consistently associated with the nontoxic acidic fractions and could even be used for a quantitative estimation (see below).

TABLE 11

CHROMATOGRAM VII

CHROMATOGRAPHY OF 400 MG. POOL INACTIVE FRACTIONS FROM CHROMATOGRAMS AS SHOWN IN TABLES 6, 9, AND SOME SLIGHTLY ACTIVE FRACTIONS FROM ANALOGUE SERIES, DISSOLVED IN 50 ML. OF PETROL ETHER ADSORBED ON 15 GM. OF MAGNESIUM SILICATE-CELITE

FRACTION	ELUTED WITH:	MG.	TOXICITY TEST			
			Amount Injected per Dose	Number of Injections	Number of Mice Dying with Typical Symptoms	Degree of Toxicity*
			mg.			
1-5	50 ml. petrol ether, each	3.8				
6-7	50 ml. petrol ether-benzene 1:1	16.3				
8	10 ml. benzene-ether 1:1	45.3	0.067 0.033	4 6	0 of 5 0 of 5	0
9	10 ml. benzene-ether 1:1	47.6				
10	10 ml. benzene-ether 1:1	21.0				
11	10 ml. benzene-ether 1:1	9.5				
12	10 ml. benzene-ether 1:1	5.7				
13	10 ml. benzene-ether 1:1	5.3				
14	10 ml. benzene-ether 1:1	3.0				
15	10 ml. benzene-ether 1:1	2.7				
16	10 ml. benzene-ether 1:1	2.4				
17	10 ml. benzene-ether 1:1	2.3				
18	10 ml. benzene-ether 1:1	2.3				
19	10 ml. benzene-ether 1:1	1.8				
20	10 ml. benzene-ether 1:1	1.7				
21	10 ml. benzene-ether 1:1	1.8				
22	50 ml. benzene-ether 1:1	6.3				
23	10 ml. ether plus 5% methanol	29.5	0.033 0.017	3 4	4 of 5 5 of 5	++
24	10 ml. ether plus 5% methanol	59.8				
25	10 ml. ether plus 5% methanol	41.1	0.017 0.008	2 2	5 of 5 3 of 5	+++
26	10 ml. ether plus 5% methanol	14.8				
27-33	10 ml. ether plus 5% methanol, each	36.4				
Recovered		360.4 = 90%				

* See footnote to table 5.

Example 2: Fraction of mild toxicity and lower acidity (fraction 23, table 11):
Melting point: 48° to 50°C.
Acidity: 6.1 mm.[3] 0.100 N sodium methylate per mg.

TABLE 12

CHROMATOGRAM VIII

CHROMATOGRAPHY OF 402 MG. POOL TOXIC FRACTIONS FROM CHROMATOGRAMS AS SHOWN IN TABLES 6, 7, AND 9, DISSOLVED IN 50 ML. OF PETROL ETHER ADSORBED ON 15 GM. OF MAGNESIUM SILICATE-CELITE

FRACTION	ELUTED WITH:	MG.	TOXICITY TEST			
			Amount Injected per Dose	Number of Injections	Number of Mice Dying with Typical Symptoms	Degree of Toxicity*
			mg.			
1-3	50 ml. petrol ether, each	traces				
4	10 ml. petrol ether-benzene 1:1	5.9				
5	10 ml. petrol ether-benzene 1:1	2.5				
6	10 ml. petrol ether-benzene 1:1	1.1				
7	10 ml. petrol ether-benzene 1:1	0.4				
8	10 ml. petrol ether-benzene 1:1	0.4				
9	10 ml. petrol ether-benzene 1:1	0.4				
10	10 ml. petrol ether-benzene 1:1	0				
11	10 ml. petrol ether-benzene 1:1	0.2				
12	10 ml. petrol ether-benzene 1:1	0.6				
13	10 ml. petrol ether-benzene 1:1	0.7				
14	10 ml. benzene-ether 1:1	0.6				
15	10 ml. benzene-ether 1:1	0.8				
16	10 ml. benzene-ether 1:1	1.0				
17	10 ml. benzene-ether 1:1	0.5				
18	10 ml. benzene-ether 1:1	39.5	0.007	1	5 of 5	+++
			0.0055	1	5 of 5	
			0.0036	4	2 of 5	
19	10 ml. benzene-ether 1:1	69.3				
20	10 ml. benzene-ether 1:1	16.4				
21	10 ml. benzene-ether 1:1	7.1				
22	10 ml. benzene-ether 1:1	5.2				
23	10 ml. benzene-ether 1:1	2.7				
24	10 ml. ether plus 5% methanol	2.3				
25	10 ml. ether plus 5% methanol	2.1				
26	10 ml. ether plus 5% methanol	1.9				
27	10 ml. ether plus 5% methanol	5.1				
28	10 ml. ether plus 5% methanol	51.2				
29	10 ml. ether plus 5% methanol	79.1	0.01	1	5 of 5	++
			0.005	5	0 of 5	
30	10 ml. ether plus 5% methanol	25.6				
31	10 ml. ether plus 5% methanol	9.8				
32	10 ml. ether plus 5% methanol	5.8				
33	10 ml. ether plus 5% methanol	5.0				
34	10 ml. ether plus 5% methanol	4.7				
35	10 ml. ether plus 5% methanol	3.3				
36	10 ml. ether plus 5% methanol	3.2				
37	10 ml. ether plus 5% methanol	2.4				
38	10 ml. ether plus 5% methanol	2.0				
39	10 ml. ether plus 5% methanol	1.8				
40	10 ml. ether plus 5% methanol	1.1				
41	10 ml. ether plus 5% methanol	0.6				
42	10 ml. ether plus 5% methanol	0.8				
43	10 ml. ether plus 5% methanol	0.7				
44	100 ml. ether plus 10% methanol	19.0				
Recovered		382.8 = 95%				

* See footnote to table 5.

Infrared spectrum (figure 2): The spectrum showed absorptions at 3,350, 3,050, 2,910, 2,850, 2,650; a strong absorption between 1,725 and 1,680, with a distinct shoulder at 1,680; absorptions at 1,470, 1,380, and a complex system of absorptions between 1,300 and 1,000; a weak band at 800 and a strong CH_2 band at 720 cm.$^{-1}$. The strong bands at 2,910, 2,850, 1,470, and 720 are the CH_2 bands already mentioned in the foregoing example.

Example 3: Purified fraction of high toxicity (fraction 18, table 12):

Melting point: 43° to 44°C.

Acidity: 1.1 mm.[3] 0.100 N sodium methylate per mg.

Infrared spectrum (figure 3): The spectrum showed a broad band at 3,350, assigned to bonded alcoholic —OH; bands at 3,050, 2,910, 2,850 (CH_2); and a strong C=O absorption between 1,725 and 1,710. This suggested an asymmetric doublet with one maximum at 1,725 cm^{-1} and a second of somewhat greater intensity at 1,710; a broad band (COO^-) between 1,625 and 1,550; bands at 1,515 (Na^+), 1,470 (CH_2), 1,380 (CH_3), 1,260; a broad system between 1,100 and 1,000, assigned to C—O stretching; and two strong bands at 800 and 720 cm.$^{-1}$.

Correlation between infrared spectrum and toxicity: It is shown in figures 1 and 3 that the spectra of the nontoxic and the highly toxic material are different.

The spectrum which was representative for the purified acidic fractions (figure 1) showed a striking similarity to the spectrum of a sample of mycolic acid prepared by R. J. Anderson[10] (figure 4). It contained all the bands of mycolic acid at the same positions and with comparable intensity, i.e., the bands at 3,500, 2,910, 2,850, 2,650, 1,705, 1,680, 1,470, 1,380, 1,300, 1,205, 1,130, 1,100, 1,017, 962, and 720 cm^{-1}. The only noticeable differences were two additional bands at 1,260 and 800 cm^{-1}. These two absorptions were always encountered together, but they appeared with varying intensities in both toxic and nontoxic fractions.[11] These spectra, which are typical representatives of approximately 40 spectra of different samples of varying degrees of toxicity and acidity, allow the following conclusions. In every instance, toxicity was associated with three characteristic absorptions:

1. At 3,350 cm.$^{-1}$, a broad band assigned to bonded alcoholic —OH.
2. At 1,725 to 1,710, a band indicating an asymmetric doublet with one maximum at 1,725 and a second of somewhat greater intensity at 1,710 cm.$^{-1}$. Absorption in this range is caused by C = O stretching.
3. At 1,100 to 1,000 cm.$^{-1}$, a broad band system assigned to C = O stretching.

As the toxicity diminished, the intensity of these bands decreased. They were completely lacking in nontoxic preparations. On the other hand, the bands at 3,500 and 1,680 cm^{-1}, which were typical of mycolic acid, appeared with increasing strength in less toxic fractions. Approximate quantitative correlations between toxicity and absorption intensities at characteristic wave lengths in a number of different preparations are given in table 10. The C=O absorptions of the active and inactive fractions overlapped, which rendered the distinction somewhat

[10] A sample of mycolic acid was kindly supplied by Dr. R. J. Anderson, Department of Chemistry, Yale University, New Haven, Conn.

[11] It was later found that these two bands are due to traces of silicone grease, which was used for greasing the stopcock of the chromatographic column.

difficult. It can easily be recognized, however, that with increasing toxicity the absorption maximum shifted from 1,680 to 1,700 toward 1,710 to 1,725, which

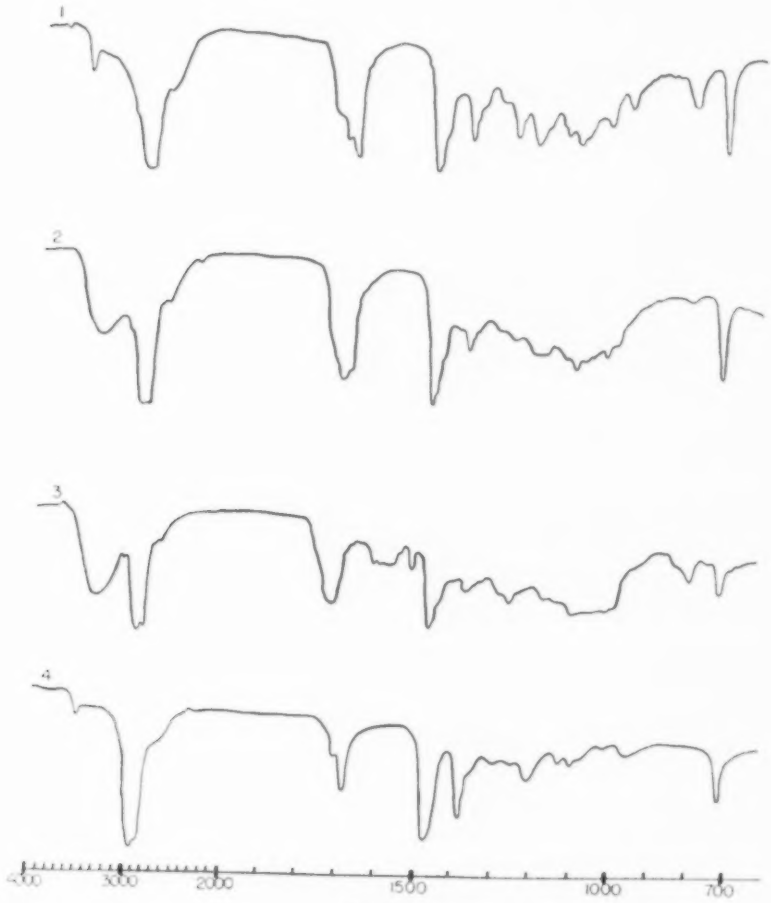

PLATE I

FIG. 1. Nontoxic acidic fraction (table 11, fraction 8).
FIG. 2. Acidic fraction of weak toxicity (table 11, fraction 23).
FIG. 3. Highly toxic fraction containing only traces of acidity (table 12, fraction 18) The sample was neutralized with sodium methylate.
FIG. 4. Mycolic acid (prepared by R. J. Anderson).

rendered the shape of the C=O absorption distinctly different in active and in inactive fractions.

Thus, a comparison between infrared spectra and toxicity of the different frac-

tions shows that two spectroscopically different components were gradually separated in the course of successive chromatographies. One of the components is nontoxic and its infrared spectrum is very similar to the one of mycolic acid, while the spectrum of the other product is markedly different.

Relation of toxicity to acidity: Another distinction between toxic and nontoxic fractions is their degree of acidity. The correlation between quantitative data on acidity and toxicity in a number of various fractions showed that the preparations concerned always represented mixtures of nontoxic acidic, and of toxic nonacidic, compounds. This evidence was brought forward by a method independent of the spectroscopic analysis.

There was a quantitative inverse relationship between acidity and toxicity as seen in table 13. Repeated injections of 100 γ of the most acidic fractions into mice did not produce any signs of toxicity, while the fractions containing only traces of acidity killed mice in amounts of only 4 to 7 γ. Thus, the fact that during purification the acidic material can be freed from any detectable toxicity and

TABLE 13

QUANTITATIVE ESTIMATION OF ACIDITY AND TOXICITY OF VARIOUS FRACTIONS

FRACTION NUMBER	ACIDITY MM.[3] 0.100 N CH_3ONa/MG	TOXIC THRESHOLD DOSE IN γ[*]	ESTIMATED INTENSITY INFRARED ABSORPTION BAND 1,580 CM.$^{-1}$	INFRARED SPECTRUM
Table 11, No. 8	6.8	>100	++++	1
23	6.1	15	+++	2
25	4.5	7.5	++	not shown
Table 12, No. 29	3.1	6	+	not shown
PP	2.0	5.0	±	6
18	1.1	4.0	±	3

[*] Threshold dose in γ which after 1 to 4 injections is lethal for at least 50 per cent of the mice.

reaches, at the same time, a limit value of acidity corresponding to an equivalent weight of 1,300 to 1,400 permits the following conclusions:

The original mixture contains two main components:

(*1*) a nontoxic acid with an equivalent weight of approximately 1,300 (or several closely related acids of similar equivalent weight)

(*2*) a toxic nonacidic material which could not yet be obtained without traces of acidic material

The other possibility that the toxic component is an acid of very high molecular weight (calculated from titration to be approximately 10,000) is not very likely because the molecular weight, determined by the method of Rast, gave a value of approximately 1,250 (see below).[12]

[12] As pointed out by Asselineau, Lederer, and Sorkin (personal communications), melting points of tuberculolipids determined by the cryoscopic method of Rast with camphor are likely to be erroneous by as much as plus-minus 30 per cent. Despite such possible errors, molecular weights of approximately 10,000 can probably be excluded.

Analytical Data of a Batch of Purified Toxic Material

The material (PP) used for analysis was a pool of toxic fractions (table 12, fractions 18 through 20). The fractions were dissolved in ether, the slightly yellow solution filtered through cotton, acidified with glacial acetic acid in order to free it from traces of salt, and precipitated by adding two parts of methanol. The precipitate was collected on filter paper, washed thoroughly with methanol, and dried in a vacuum desiccator over P_2O_5. It formed a white powder, melting point 42° to 44°C. The acidity of this pool was 2.0 mm^3. 0.100 N CH_3ONa per mg., corresponding to a content of 26 per cent acid of molecular weight 1,300.

Toxicity: Four injections of 10 γ each caused death of 5 of 5 mice, four injections of 5 γ were lethal for 3 of 5 mice; all animals died with characteristic pulmonary hemorrhages.

Organic quantitative micro-analysis[13] gave the following data:

1) Carbon hydrogen
3.557 mg. of substance gave 10.305 mg. of CO_2 and 4.110 mg. of H_2O
2.762 mg. of substance gave 8.005 mg. of CO_2 and 3.250 mg. of H_2O
found: C 79.01% H 12.93%
79.04% 13.17%

2) Nitrogen (Dumas)
3.235 mg. of substance gave 0.048 cc. of N_2 (t = 26° C.; p = 761 mm.)
6.950 mg. of substance gave 0.089 cc. of N_2 (t = 27.5° C.; p = 768 mm.)
found: N 1.20%
1.46%

3) —O CH_3 (Zeisel)
0.452 mg. of substance used 0.33 ml. of 0.02 N $Na_2S_2O_3$ solution
found: —O CH_3 0.6% (trace)

4) Active hydrogen (reduction with lithium aluminum hydride)
42.348 mg. of substance gave 2.77 cc. of H_2 (t/p = 26°/754 mm.)
found: H^+ 0.26%

5) Molecular weight (Rast)
0.452 mg. of substance in 4.420 mg. of camphor gave Δ t 3.1°
found: MW 1,254 ± 10%[13]

The micro-analytical test for phosphorus was negative. A qualitative spectrographic analysis[14] showed presence of phosphorus only in traces, estimated range between 0.1 and 0.01 per cent, which rules out phosphorus as part of the molecule.

Ultraviolet spectrum: An ultraviolet spectrum of the material PP was taken in the Beckman spectrophotometer. The concentration of the sample was 0.5 mg. per cc. in iso-octane. The range tested was 320 to 215 mμ. No significant absorption maxima could be detected, but there was a strong end absorption between 225 to 215 mμ. The following three values are representative:

λ = 300 mμ log ϵ = 2.14
λ = 230 mμ log ϵ = 2.76
λ = 215 mμ log ϵ = 3.02

The calibration of the apparatus was checked with a test sample of benzoic acid, which gave exactly the expected values.

[13] Analyses by Schwarzkopf Microanalytical Laboratory, Queens, New York.
[14] American Cyanamid Company, Stamford, Conn., Drs. L. Dutton and R. C. Gore.

Chemical Treatment Resulting in Loss of Toxicity[15]

Isolation and properties of the reduced compound after treatment with lithium aluminum hydride: The reduced product was obtained in butyl ether solution as the lithium aluminum salt resulting from the determination of active hydrogen. After addition of 2 N sulfuric acid, it was extracted with ethyl ether, the combined ether layers were washed with water until the wash water was neutral to litmus and was distilled off under reduced pressure. The residue was dissolved in ether, where it formed an opaque solution. After standing, a gelatinous precipitate separated, which was filtered off. The clear ether solution was evaporated to dryness. It yielded a white powder, melting point 53° to 56°C. Approximately 50 per cent of the amount used for reduction was recovered in the ether solution. The water extract and the distillate were not further investigated.

The infrared spectrum of the ether-soluble reduction product (figure 5) differed from that of the parent substance (figure 6) in only one respect; the strong C=O absorption at 1,725 to 1,710 cm.$^{-1}$ had completely disappeared. The weak absorption at 1,680 cm.$^{-1}$ in figure 6 which was due to the acidic impurity of the parent substance had disappeared, indicating that all C=O groups were reduced.

The reduced compound showed no toxicity for mice. Six injections of 0.1 mg. each did not cause any pathologic symptoms. Since the C=O absorption at 1,725 to 1,710 cm.$^{-1}$ was found to be characteristic for the toxic material, the loss of toxicity in the reduced compound indicated that the C=O group was essential for toxic properties.

Saponification: As pointed out in the previous paper in this series (1) and from previous unpublished observations, it seemed that toxicity is greatly reduced after saponification. This fact is further illustrated by the following experiment:

Twelve milligrams of pooled active fractions (table 12, starting material) were dissolved in a mixture of 3 ml. of diethyleneglycol and 1 ml. of H_2O containing 0.05 gm. of KOH. The lipid material dissolved upon heating, forming a clear yellow solution. After heating for approximately one-half hour, 10 ml. of water were added, acidified with HCl, and extracted with ether. The combined ether layers were washed with water and dried over anhydrous magnesium sulfate. After evaporation of the ether, about 10 mg. of a white powder were recovered, melting point 48° to 54°C.

The acidity before treatment was 2.3 mm.^{3} 0.100 N CH_3ONa per mg. After saponification, it was 5.8 mm.^{3} 0.100 CH_3ONa per mg. This corresponded to an equivalent weight of approximately 1,780. The increased acidity indicated that an acid had been liberated.

A comparison between the infrared spectra of the ether-soluble saponification product (figure 7) with that of the parent substance (figure 8) showed that the C = O absorption of the saponified substance shifted from 1,725 to 1,705 cm.$^{-1}$ with the formation of a strong band at 1,680, which had been found to be typical for mycolic acid and for the nontoxic fractions. Furthermore, the strong bonded —OH absorption at 3,350 cm.$^{-1}$ of the original material which was always present in the toxic material had become very weak, while the unbonded —OH band at 3,500 cm.$^{-1}$ which was also present in mycolic acid, on the other hand, appeared in the saponified product. The equivalent weight of 1,780 determined by titration indicated that the saponification was not complete because less than one equiva-

[15] Although the following reactions were done on a toxic material which was not chemically pure, they are reported because the results seem to be of interest and indicate possibilities for further chemical studies.

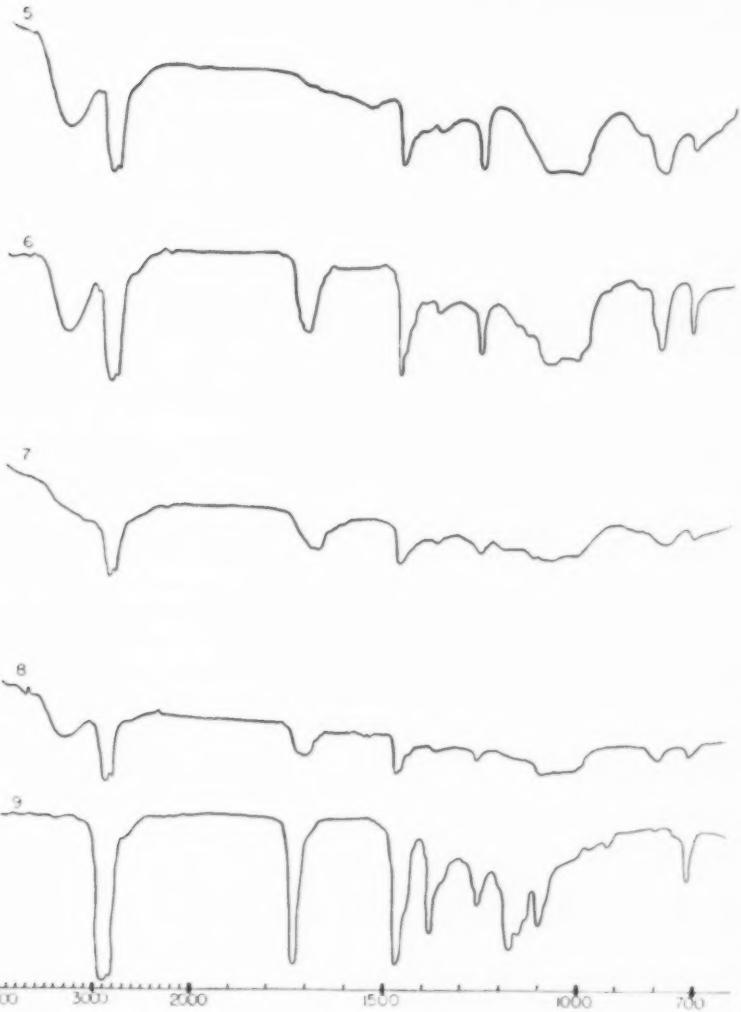

PLATE II

FIG. 5. Toxic fractions (pool "PP") after reduction with lithium aluminum hydride.
FIG. 6. Toxic fractions (pool "PP") before reduction.
FIG. 7. Pool of toxic fractions after saponification.
FIG. 8. Pool of toxic fractions before saponification.
FIG. 9. Neutral compound (melting point 30°C.), probably an ester of phthiocerol with mycocerosic acid.

lent of acid was liberated. The fact that, although a loss of toxicity had occurred, the saponified material was still slightly toxic (table 14) points to the same direction.

It can thus be concluded that saponification destroys activity by attacking the same C=O group which appears to be essential for toxicity.

TABLE 14

THE EFFECT OF SAPONIFICATION ON THE TOXICITY OF A POOL OF TOXIC FRACTIONS

	γ INJECTED PER DOSE	NUMBER OF INJECTIONS	NUMBER OF MICE DYING WITH TYPICAL SYMPTOMS	LOSS OF WEIGHT
Pooled toxic fractions	20	3	5 of 5	yes
Pooled toxic fractions	10	2	3 of 5	yes
Pooled after saponification	100	5	0 of 5	yes
Pooled after saponification	20	5	0 of 5	no

DISCUSSION

The present study is concerned with the isolation of a toxic material from the "purified wax" of virulent tubercle bacilli. The constituents of the "purified wax" have been the subject of numerous papers in the past. However, none of the purified components described by previous investigators has the properties of the toxic material described in this series of papers. There are several reasons which account for the previous failure to detect the presence of the toxic material in the "purified wax": (*1*) It constitutes only about 1 per cent of the "purified wax"; (*2*) it is destroyed by hydrolysis; and (*3*) its isolation and purification were only possible with the aid of a sensitive biologic test.

Earlier investigations on the composition of the "purified wax" (for a recent review see Lederer (11)) led to the isolation of three components:

(*a*) a lipopolysaccharide (6, 7)

(*b*) an ester of phthiocerol with a fatty acid (7, 9), the latter identified as mycocerosic acid (7, 10)

(*c*) free mycolic acid (7, 9)

During the fractionation of the "purified wax" in the present study, three main components were isolated, the properties of which are similar to those of the three components just mentioned.

The similarity of the neutral material, melting point 30°C., to the ester of phthiocerol and mycocerosic acid has been mentioned before. The infrared spectrum (figure 9) shows a series of well-defined bands at 2,900, 1,731, 1,462, 1,381, 1,352, 1,260, 1,173, 1,152, 1,133, 1,098, 970, 924, 802, 755, and 720 cm.$^{-1}$. It is distinctly different from the spectra of the other components and considerably less complex. The only absorption in the C=O region is a strong sharp band at 1,731, which is typical of esters. The absence of—OH absorption between 3,000 to 3,500 cm.$^{-1}$ suggests that both hydroxyl groups of the dialcohol phthiocerol are linked to the acid, but the analytical data published by Asselineau and Philpot and Wells give no information on this point.

There is a striking similarity between the infrared spectrum of this ester and

the spectra of the lipids extracted from H37Rv by Randall, Smith, and Nungester (12). From their method of extraction (although different from all well-known procedures described in the literature) and from the abundance of this ester in the wax of virulent tubercle bacilli, it is tempting to conclude that the ester of phthiocerol and mycocerosic acid is the prevailing component in their mixture.

Asselineau (7) and Philpot and Wells (9) claim that the "purified wax" contains free mycolic acid. This statement is based on the finding that the acidic fractions obtained by chromatography of the "purified wax" have the same melting points and equivalent weights as samples of mycolic acid isolated after saponification of "purified wax." The similarity of the two acids is further corroborated by a comparison of the infrared spectra (figures 1 and 4). The—CH_3 band at 1,380 cm.$^{-1}$, which is strong in both samples, is in agreement with the branched chain character of mycolic acid demonstrated by chemical analysis (11, 13). The sharp band at 3,500 cm.$^{-1}$, typical for unbonded—OH, was already mentioned by Asselineau (7). It is absent in anhydromycolic acid, where the—OH has been removed by dehydration (14).

The toxic material has not yet been obtained in pure form. Small amounts of acidic fractions were still adhering to the toxic component. This is possibly due to partial hydrolysis of the neutral material on the column. Nevertheless, it can safely be stated that the physical and chemical properties of the toxic component are distinctly different from the other constituents of the "purified wax." Titrations and infrared spectrum show that it is not an acid. A simple experiment underlined this fact further: It is well known that conversion of a free acid into a salt causes a shift of the acidic C=O absorption toward longer wave lengths. In the present experiment, a highly toxic sample (table 12, fraction 18) was neutralized with sodium methylate. This caused the weak C=O absorption (figure 6) due to the acid impurity to shift from 1,680 to a broad band between 1,625 and 1,550 cm.$^{-1}$, typical of ionized carboxy. Furthermore, a sodium band appeared at 1,515 cm.$^{-1}$ (figure 3). On the other hand, the strong C=O absorption at 1,725 to 1,710 cm.$^{-1}$ remained unchanged, which proved that the strong C=O band found to be characteristic for the toxic material was not due to acidic C=O absorption.

An interesting fact is the presence of nitrogen in the toxic material.[16] Nitrogen has only been found, so far, in the lipopolysaccharide fraction of the "purified wax" (15) and in the purified toxic material obtained from petroleum ether extracts of virulent tubercle bacilli (1). As hydrolysis of the toxic material liberates an acid resembling mycolic acid, and as mycolic acid is part of the structure of the lipopolysaccharide (6, 7, 11), one may speculate whether the toxic substance

[16] The possible function of nitrogen was not discussed in connection with the infrared spectra because no specific nitrogen absorption could be detected. There was no absorption in the range of the C=N stretching (1,700-1,600 cm.$^{-1}$). Absorptions due to the C—N and N—H stretching are in the same range as the corresponding C—O and O—H stretching frequencies and are therefore difficult to distinguish. Moreover, the nitrogen content of the toxic material is so low that its absorptions might well escape detection, especially in the presence of other absorbing groups.

is a precursor of the lipid part of the lipopolysaccharide linking the fatty acid to the polysaccharide part of the molecule. This would be in agreement with the fact that more toxic material is obtained from young bacterial cultures while old cultures, on the other hand, are relatively richer in lipopolysaccharide (7). The assumption would be in good agreement, too, with the observations reported in table 2, showing that in young bacterial cultures a good part of the acetone-soluble fraction of "purified wax" containing the toxic material can be extracted with petroleum ether.

SUMMARY

A toxic material was obtained from the chloroform extracts of live and heat-killed cultures of virulent tubercle bacilli, harvested at different times. It was found to be present in the acetone-soluble part of the "purified wax." The purification of the toxic material by means of chromatography is described.

The toxicity, the infrared spectra, and the available chemical data permit characterization of this bacterial constituent and its differentiation from other known constituents of the "purified wax."

The material is similar or identical to the analogous fraction isolated by petroleum ether extraction from live young cultures of tubercle bacilli.

The toxic material is closely associated with an acidic component which is probably identical with mycolic acid. In the course of chromatographic purification, it was found that with increasing toxicity of the purified fractions their acidity decreased. At the same rate, the infrared absorptions characteristic for mycolic acid disappeared from the spectrum. These observations suggest that the toxic material itself is not an acid, although it was not yet possible to obtain it completely free of acid. The molecular weight of the toxic component is in the same range as that of mycolic acid. The toxic fraction contains approximately 1 per cent nitrogen (corresponding to 1 nitrogen atom per molecule). It can be reduced and hydrolyzed, both reactions resulting in a loss of toxicity.

SUMARIO

Componente Lípido Tóxico del Bacilo Tuberculoso ("Factor Cord"): II. Existencia en Extractos Clorofórmicos de Cultivos Bacterianos Jóvenes y Viejos

De los extractos clorofórmicos de cultivos vivos y matados al calor de bacilos tuberculosos virulentos, obtúvose una substancia tóxica. Encontróse presente en la porción acetonosoluble de la Cera Purificada. Descríbese la purificación de la substancia tóxica por medio de la cromatografía.

La toxicidad, los espectros infrarrojos y los datos químicos disponibles permiten caracterizar ese componente bacteriano y diferenciarlo de otros componentes conocidos de la Cera Purificada.

La substancia es semejante o idéntica a la fracción análoga aislada por extracción con éter de petróleo de cultivos jóvenes vivos de bacilos tuberculosos.

La substancia tóxica está íntimamente asociada con un componente acidógeno que es probablemente idéntico al ácido micólico. En el transcurso de la purificación cromatográfica, se descubrió que, al aumentar la toxicidad de las fracciones purificadas, disminuía su acidez. Con la misma velocidad, desaparecían del espectro las absorciones infrarrojas que caracterizan al ácido micólico. Esas observaciones indican que la substancia tóxica misma

no es un ácido, aunque no ha sido posible obtenerla aun completamente acidopriva. El peso molecular del componente tóxico queda en la misma escala que el del ácido micólico. La fracción tóxica contiene aproximadamente 1 por ciento de nitrógeno (correspondiendo a 1 átomo de nitrógeno por molécula). Puede reducirse e hidrolizarse, dando ambas reacciones por resultado pérdida de toxicidad.

RESUME

Un composant toxique lipidique du bacille tuberculeux ("Cord Factor"): II. Présence dans les extraits de cultures bactériennes jeunes ou vieilles traitées par le chloroforme

Une substance toxique a été extraite par le chloroforme de cultures de bacilles tuberculeux virulents, vivantes ou tuées, à différents stades de leur croissance. Elle a été trouvée dans la fraction soluble dans l'acétone de la cire purifiée. La purification de la substance toxique par la méthode chromatographique est décrite.

La toxicité, le spectre infra-rouge et les données chimiques permettent d'caractériser ce composant bactérien et de le différencier des autres composants connus de la cire purifiée.

Cette substance est similaire ou identique à la fraction analogue isolée de jeunes cultures vivantes du bacille de Koch, par extraction avec l'éther de pétrole.

Cette substance toxique est étroitement associée à un composant acide qui paraît semblable à l'acide mycolique. Au cours de la séparation par la méthode chromatographique, on a trouvé que l'acidité diminuait à mesure que la toxicité de la fraction purifiée augmentait. Les bands d'absorption dans l'infra-rouge, qui caractérise l'acide mycolique, disparaissait du spectre en même temps. Ces observations suggèrent que la substance toxique elle-méme, tout en n'étant pas complètement libérée de l'acide associé, n'est pas un acide. Le poids moléculaire du composant toxique est dans la gamme de ceux de l'acide mycolique. La fraction toxique contient approximativement 1% d'azote (soit 1 atome d'azote par molécule). Cette fraction peut être réduite ou hydrolysée; l'un et l'autre de ces deux réactions détermine une perte de toxicité.

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A TOXIC LIPID COMPONENT OF THE TUBERCLE BACILLUS ("CORD FACTOR")[1]

III. Occurrence and Distribution in Various Bacterial Extracts

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INTRODUCTION

Tubercle bacilli contain a lipid component which is characterized by a high degree of toxicity. This material was termed "cord factor."[2] It was first obtained from young bacterial cultures by superficial extraction with petroleum ether (1). A number of observations reported and discussed in previous papers (2, 3) suggest that the material extracted in this manner originates from the surface of the bacterial cell. With the method of extraction used, it could be obtained only from young cultures of "cord-forming" (4) organisms (virulent strains and BCG). Subsequent studies showed that the petroleum ether extracts are mixtures of various compounds, only one of which is toxic (5). The toxic component was separated from the other remaining fractions and obtained in concentrated form (6).

The present experiments were begun in the summer of 1950 with the intention of searching for a material of comparable toxicity in preparations which were obtained in the course of a chemical investigation of various bacterial lipids at the Institut de Biologie Physico-Chimique in Paris. The extraction procedure used in the Paris laboratory is a slight modification of the scheme elaborated by R. J. Anderson and his associates (7). It has been described before (8, 9) and it is sufficient at present to state that the following fractions were obtained:

From the alcohol-ether extract of tubercle bacilli:

1. The acetone-soluble fat
2. The phosphatides (insoluble in boiling acetone)
3. The wax A (soluble in boiling acetone)

From the chloroform extract of bacilli after extraction with alcohol-ether:

4. The wax B (soluble in a mixture of methanol and ether)
5. The wax C (soluble in hot acetone)
6. The wax D (insoluble in boiling acetone)

[1] From the Institut de Biologie Physico-Chimique, Paris, France; and the Public Health Research Institute of The City of New York, Inc., New York, New York.

[2] The term "cord factor" had been suggested by one of the writers (H. B.) (1) because the material in question was obtained from "cord-forming" strains of tubercle bacilli (virulent organisms and BCG) and seemed to be responsible, at least partly, for the characteristic parallel arrangement of the organisms. One of the other writers (E. L.) (16, 17) took issue with this appellation and suggested that it be replaced by two terms; one for the toxic factor, the other for the component responsible for the formation of "cords." In the meantime, the two writers became associated and suggest that, as long as it cannot be replaced by an unequivocal chemical formula, the term "cord factor" be retained as what it was always meant to be: a short convenient designation, intending no more than to denote a characteristic bacterial component which was first extracted from "cord-forming" cultures of tubercle bacilli.

More than 70 samples of such fractions and derivatives were investigated in the present study. They came from various strains of bacilli and from cultures of different ages. The toxicity for mice which was previously found to be characteristic for "cord factor" was used as a screening test. While these experiments were still going on, a toxic component similar, perhaps identical, to the one present in petroleum ether extracts of young cultures was isolated at the New York laboratory from older growths of tubercle bacilli (10). As will be seen from the present and the foregoing publications, the results of the two series of experiments are in good agreement. The toxic material was found in analogous fractions from various bacterial strains extracted by different techniques.

EXPERIMENTAL

Bacteria: The organisms used in these experiments were all cultured at the Institut Pasteur in Paris. They were grown on Sauton medium and harvested at different times. After filtration, the bacteria were immediately placed in a mixture of alcohol-ether 1:1. The extraction with this mixture was repeated five to six times at room temperature until the solvent contained no more material. The cultures were then extracted with chloroform at room temperature. Detailed descriptions of the extraction procedure have previously been published (11, 12).

The following bacterial strains were used:

Test, Brévannes, H37Rv: virulent strains of the human type

A streptomycin-resistant variant of the *H37Rv* strain

Marmorek, a bovine strain of somewhat attenuated virulence

BCG, the strain which is used at the Pasteur Institute for the production of vaccine

Biologic assay: The method of the screening test has been described (3). This paper should be consulted for all details. All tests were done with C57 black mice.

The Distribution of the Toxic Component Among Various Lipid Fractions

The results of the toxicity studies in mice are summarized in table 1. All mice which died showed characteristic pulmonary hemorrhages. It can be seen in the table that characteristic toxicity was found in extracts of the five strains, *Brévannes*, *Marmorek*, *H37Rv*, a streptomycin-resistant variant of *H37Rv*, and *BCG*. The toxic component was mostly present in the wax C fractions, but also some of the wax D fractions were active. In the light of the results reported in the previous papers in this series (6, 10), it appears that during the fractionation procedure which is used in Paris the active component is only partly extracted by boiling acetone and is therefore found in the wax C as well as the wax D fractions. Of the human strain *Test*, the acetone-soluble fat, the wax C as well as the wax D were inactive.

At a certain stage of the purification process, toxicity was always found in the fractions which contained acidic compounds of high molecular weight similar to mycolic acid. Consequently, the toxicity of the following branched-chain acids was determined:

(*a*) saturated β-hydroxy acids: synthetic 2-hexadecyl,3-hydroxy-eicosanoic acid $C_{36}H_{72}O_3$ (13) and the natural α and γ mycolic acids of the *Brévannes* strain (12)

(*b*) an unsaturated β-hydroxy acid: synthetic methyl 2-(7'-tetradecene)3-hydroxy,11-octadecenoate, $C_{33}H_{64}O_3$[3]

[3] Unpublished experiments by J. Polonsky, J. Pudles, and E. Lederer.

TABLE 1

THE DISTRIBUTION OF TOXIC MATERIAL AMONG VARIOUS FRACTIONS FROM DIFFERENT BACTERIAL STRAINS

BACTERIAL STRAIN FROM WHICH PREPARATION WAS OBTAINED	AGE OF CULTURE	CHEMICAL DESIGNATION OF PREPARATION	AMOUNT INJECTED PER DOSE	NUMBER OF INJECTIONS	NUMBER OF MICE DYING WITH TYPICAL SYMPTOMS	DEGREE OF TOXICITY
	days		*mg.*			
Test	42	Wax C	0.1	6	0 of 5	0
Test	42	Wax D	0.1	5	0 of 5	0
Brévannes	28	Wax C	0.1	3	6 of 6	+++
Brévannes	28	Wax D	0.1	4	4 of 5	++
Brévannes	28	α-Mycolic acid	0.1	5	0 of 5	0
Brévannes	28	Phosphatide	0.1	5	0 of 4	0
Brévannes	28	Polysaccharide*	0.1	5	0 of 5	0
Brévannes	28	Fatty acids (mixture)†	0.1	5	0 of 5	0
H37Rv	42	Wax B	0.1	5	2 of 5	+
H37Rv	42	Wax C	0.1	4	5 of 6	++
H37Rv	28	Wax D	0.1	4	1 of 5	+
H37Rv, streptomycin-resistant	28	Wax C	0.1	2	5 of 5	
H37Rv, streptomycin-resistant	28	Wax C	0.05	3	3 of 5	+++
H37Rv, streptomycin-resistant	28	Wax C	0.01	5	0 of 5	
H37Rv, streptomycin-resistant	28	Wax D	0.1	5	4 of 5	++
Marmorek	4	Wax D	0.1	4	2 of 5	+
Marmorek	28	Wax D	0.1	2	4 of 5	++
Marmorek	28	Wax D	0.05	5	0 of 5	
Marmorek	28	Phthiodiolone, $C_{34}H_{66}O_3$† (melting point 80°C.)	0.1	4	0 of 5	0
BCG	4	Wax C	0.1	4	0 of 5	0
BCG	4	Wax D	0.1	3	2 of 5	+
BCG	4	Wax A	0.1	5	0 of 5	0
BCG	5	Wax D	0.1	2	5 of 5	+++
BCG	8	Wax D	0.1	4	4 of 4	++
BCG	15	Wax D	0.1	3	5 of 5	+++
BCG	15	Wax D	0.05	6	3 of 5	
BCG	15	Wax C	0.1	2	5 of 5	
BCG	15	Wax C	0.05	3	5 of 5	+++
BCG	15	Wax C	0.025	4	0 of 5	

* From saponified wax D.

† Unpublished experiments by H. Demarteau-Ginsburg and E. Lederer.

(*c*) an α,β-unsaturated acid: 2-(7′-hexadecene),2,11-eicosadienoic acid, $C_{38}H_{66}O_2$[3]

None of these substances was toxic. Likewise, no toxicity was found in a polysaccharide and in low molecular weight fatty acids obtained after saponification of the wax D fractions from the *Brévannes* and *BCG* strains.[4]

[4] Analogous saponification products are also reported nontoxic in the foregoing paper (10).

DISCUSSION

Since the beginning of the studies on the nature of "cord factor" and the possible role of the toxicity of this material during infection, an important question has been whether the toxic compound occurs exclusively in petroleum ether extracts from young cultures of virulent organisms or whether it can also be found in other fractions of the tubercle bacillus. It was not too surprising that a similar compound should not previously have been described among the numerous substances isolated from tubercle bacilli because it appears well established by now that the toxic constituent represents but a very small fraction of the total lipids. Its physical properties are so similar to those of other lipid components that its presence in crude extracts cannot be easily detected unless a sensitive method of assay is used to locate it at each step of the chemical fractionation. Moreover, the toxic compound appears to be sensitive to hydrolysis, a procedure often employed in isolating lipids from *Mycobacteria*.

Evidence has been presented earlier that the material extracted with petroleum ether accumulates at the surface of the bacterium. Under favorable conditions, it is released into the surrounding medium (3). Nevertheless, it was not unlikely that a thorough break-up of the bacterial cell would yield such additional material. As long as only crude extracts were available for study, it was not possible to differentiate the material obtained by petroleum ether extraction from other bacterial constituents which had been studied more carefully chemically, such as lipopolysaccharides or the mycolic acids. This question has now been clarified by the experiments reported in the present series of papers (6, 10). It was shown that a toxic component is present, not only in petroleum ether extracts from young cultures of tubercle bacilli, but also in similar extracts from older cultures. The component is likewise present in chloroform extracts from bacteria which were previously extracted with petroleum ether, and in comparable fractions from younger and older cultures. It cannot be stated at the present time, however, whether the toxic fractions obtained from all of these sources are identical. It has been established, for example, that the mycolic acids isolated from different bacterial strains vary slightly in their molecular structure (8, 9, 11, 16, 17). Nevertheless, it is idle to speculate whether this is also true of the toxic principle before more is known of its chemical composition. Biologically, the various toxic products are indistinguishable with the methods used so far.

It is obvious from the data presented in the present paper that the yield of active material was irregular and that it did not always occur in the same fractions. Since, at this stage of fractionation, isolation of different compounds was based on their different solubility in organic solvents, the findings suggest that this method is not sensitive enough to separate the toxic principle from the remaining chemically similar substances. On the other hand, the appreciable amounts of toxic material found in some of these fractions promise to open new avenues of approach to obtaining considerably better yields of such material for further biologic and chemical studies.

Only experiments with fractions from bacterial strains which, *in vitro*, form colonies of a type which is characteristic of virulent organisms, i.e., "cord form-

ers" (4) are reported in the present paper. Despite their varying pathogenicity, all of these strains share the ability to multiply in a susceptible host. This includes *BCG*, which also has other properties in common with fully virulent strains. In both the neutral red (14) and the methylene blue tests (15) it acts like a virulent strain, which is in contrast to avirulent variants of the tubercle bacillus whose cultural, tinctorial, and pathogenic properties differ strongly from the ones of "cord-forming" strains. Preliminary experiments showed that extracts from an avirulent strain (H37Ra) were devoid of the toxicity which characterizes products from virulent organisms, but the experiments have to be pursued further before definite conclusions can be drawn.

Since some of the tested wax D fractions, which are essentially lipopolysaccharides, were found to contain varying amounts of toxic material, the dioxane-soluble part of another lipopolysaccharide from tubercle bacilli ("Pmko" (18)) was also tested. This fraction was kindly supplied by Dr. N. Choucroun. No toxicity was found in this fraction.

SUMMARY

The characteristic toxicity of "cord factor" was found in two of the wax fractions obtained from chloroform extracts of various strains of virulent tubercle bacilli and of the *BCG* strain. By this method of extraction, toxic fractions were obtained, not only from young cultures, but also from four- and six-week-old bacterial cultures. Synthetic branched chain α-alkyl, β-hydroxy acids, and bacterial mycolic acids were inactive.

SUMARIO

Componente Lípido Tóxico del Bacilo Tuberculoso ("Factor Cord"): II. Existencia y Distribución en Varios Extractos Bacterianos

La típica toxicidad del factor "cord" fué descubierta en dos de las fracciones céreas obtenidas de extractos clorofórmicos de varias cepas de bacilos tuberculosos virulentos y de la cepa BCG. Con ese método de extracción, obtuviéronse fracciones tóxicas, no sólo de cultivos jóvenes, sino también de cultivos de cuatro y de seis semanas. Se mostraron inactivos los ácidos α-alquil, β-hidroxílicos sintéticos de cadena ramificada y los ácidos micólicos bacterianos.

RESUME

Un composant toxique lipoïdique du bacille tuberculeux ("Cord Factor"): II. Présence et distribution dans divers extraits bactériens

La toxicité caractéristique du "cord factor" a été observée dans deux des fractions de cire obtenues par l'extraction par le chloroforme à partir de divers souches de bacilles tuberculeux virulents et de la souche BCG. Cette méthode d'extraction a permis d'obtenir les fractions toxiques, non seulement de cultures jeunes, mais aussi de cultures bactériennes âgées de six semaines. Les acides synthétiques à chaînes latérales α-alkyl, β-hydroxy et les acides mycoliques bactériens se sont montrés inactifs.

Acknowledgments

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Case Report

CASEATION NECROSIS IN HUMAN BRUCELLOSIS[1]

Report of a Case

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INTRODUCTION

Since 1897, when Hughes (1) published his monograph entitled, *Mediterranean, Malta, or Undulant Fever*, information on the many aspects of brucellosis has continued to come at a steady but often discouragingly slow rate. Although the work of the Mediterranean Fever Commission, under the chairmanship of David Bruce, during the early years of this century brought under control for the first time the British Army's chief medical problem on the island of Malta, brucellosis continues to perplex the clinician and medical scientist of today. It is the purpose of this paper to consider just one of the interesting and intriguing aspects of the brucellosis picture. The study throws no new light on the over all problem other than to stress the need for an appreciation of the body's frequently uniform reaction to injury regardless of the invading agent. Failure to appreciate this may lead to clinical error.

CASE REPORT

A 21 year old Negro male soldier was admitted to a general hospital on May 23, 1951, with the chief complaints of vomiting and diarrhea. He had been in good health until May 21, 1951, when he developed vomiting, followed in twenty four hours by dark, non bloody, loose stools with associated cramping nonradiating low abdominal pain, malaise, and a feverish sensation without chills. The patient had been aware of an asymptomatic generalized lymphadenopathy for several weeks prior to this illness. For almost a year preceding the present illness the patient had been employed in a Chicago meat packing plant where he had engaged in the slaughtering of cattle and hogs. Some three months before this hospitalization, he sustained a laceration to his lower lip when struck by the thrashings of a wounded bleeding pig. This lesion healed without incident.

Physical examination: Physical examination on admission revealed that the patient weighed 169 pounds. His temperature was 102.2 F. His pulse rate was 96 per minute; blood pressure, 120 systolic and 68 diastolic in mm. of mercury. He was a tall, slender, well-nourished Negro male who appeared acutely ill. Several nontender, freely movable, firm cervical lymph nodes, 0.5 to 2 cm. in size, were palpable. Similar lymph nodes could be felt in the supraclavicular, axillary, inguinal, and epitrochlear regions. The heart was not enlarged to palpation or percussion. The mitral first sound was accentuated and a soft, Grade II systolic murmur was heard over the basal area with transmission to the right neck vessels. Slight abdominal tenderness was elicited on deep palpation of the left lower quadrant. The liver and spleen were neither palpable nor tender. The remainder of the physical examination was normal.

Laboratory studies: A urine specimen obtained on May 25, 1951, had a specific gravity of 1.020, there was a trace of albumin, and many bacteria were present. Urinalysis two days

[1] From the Brooke Army Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas.

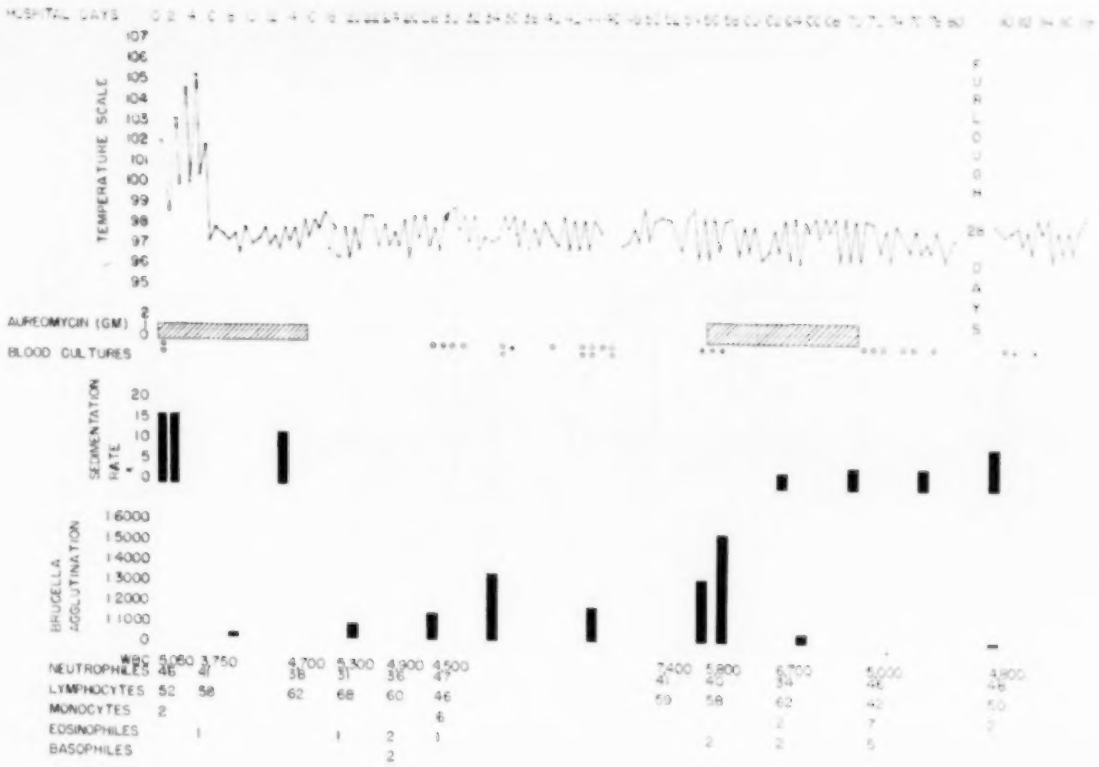

FIG. 1. This figure illustrates the course during hospitalization with regard to the temperature, erythrocyte sedimentation rate, agglutinations for brucellosis, total and differential leukocyte count, blood cultures, and treatment.

later showed one-plus albumin, many leukocytes and epithelial cells, and amorphous material per high power field. A "clean catch" urine specimen cultured on May 26, 1951, contained a nonhemolytic *Staphylococcus aureus*. This specimen contained 1 to 5 epithelial cells, 5 to 8 leukocytes, and 1 to 3 erythrocytes per high power field. On May 27, 1951, a catheterized urine specimen contained a trace of albumin, 1 to 2 epithelial cells, 1 to 2 leukocytes per high power field, and amorphous urates. No growth was obtained from this specimen on culture. Subsequent urine examinations remained normal.

The findings during the hospital course regarding the blood count, erythrocyte sedimentation rate, blood cultures, agglutinations for brucellosis, temperature, and treatment are summarized in figure 1.

Brucella suis recovered by blood cultures was highly susceptible *in vitro* to chloramphenicol, aureomycin, and streptomycin and was resistant to penicillin and sulfadiazine. An aspirate of bone marrow obtained on July 9, 1951, contained no growth at the end of thirty-

TABLE 1

TESTS OF HEPATIC FUNCTION

	DAY OF DISEASE		
	25	30	84
Serum bilirubin—direct (mg. per 100 ml.)	0.2	0.0	0.0
total	0.2	0.2	0.0
Thymol turbidity (units)	11.8	1.25	8.0
Cephalin flocculation—24 hours	1+		1+
48 hours	3+		2+
Bromsulfalein (retention at 45 minutes)	8 per cent		3 per cent
Total serum protein (gm. per 100 ml.)		8.0	8.4
Albumin		5.33	4.9
Globulin		2.67	3.5
A:G ratio		1.6:1	1.4:1
Cholesterol—total (mg. per 100 ml.)	172.0		137.0
esters			79.5
Alkaline phosphatase (Bodansky units)			0.23

four days' culture for *Brucella*, and no growth at the end of six weeks' culture for tubercle bacilli. A serologic test for syphilis, smears for malaria, stool examinations for ova, parasites, and enteric pathogens, and agglutinations for heterophil antibodies and cold agglutinins were negative. Roentgenographic examination of the chest, the upper gastrointestinal tract, and colon revealed no abnormality. An intravenous pyelogram was normal. An electrocardiogram was interpreted as normal. Intradermal tests with blastomycin, coccidioidin, and histoplasmin were negative. Tuberculin intradermal test with PPD No. 1 was one-plus in twenty-four hours and two-plus in forty-eight hours. Two weeks later a small area of ulceration was noted at the site of the PPD No. 1 injection, and culture of material from this lesion revealed a hemolytic *Staphylococcus aureus*. The results of liver function tests are shown in table 1.

Histopathologic findings: A right supraclavicular lymph node was biopsied on June 1, 1951. Pathologic examination revealed the specimen to consist of five firm, gray-white lymph nodes with yellow, granular cut surfaces. Microscopically, the lymph nodes were

poorly circumscribed and partially encapsulated. Almost the entire section was composed of large confluent masses of avascular caseation necrosis (figure 2). Scattered foci of calcification were noted. Dense bands of collagen and scattered foci of lymphocytes were identified around the margins. Occasional multinucleated giant cells of the Langhans' type were seen (figures 3 and 4). While occasional scattered epithelioid cells were noted, they were arranged in layers. At the periphery of the section, occasional lymphoid follicles were noted. Stains for acid fast bacilli were negative. Gram stains were negative.

Microscopically, the sections were those of a granuloma. The avascular caseation necrosis was suggestive of tuberculosis. While there was a paucity of Langhans' type giant cells and epithelioid cells, much as might be seen in an old healing lesion, there was no replacement fibrosis and hyalinization as might be expected with a lesion of tuberculous origin.

Pathologic examination of a specimen of liver, obtained by needle biopsy on July 20, 1951, revealed the characteristic lobular pattern. The hepatic cells were polygonal in shape

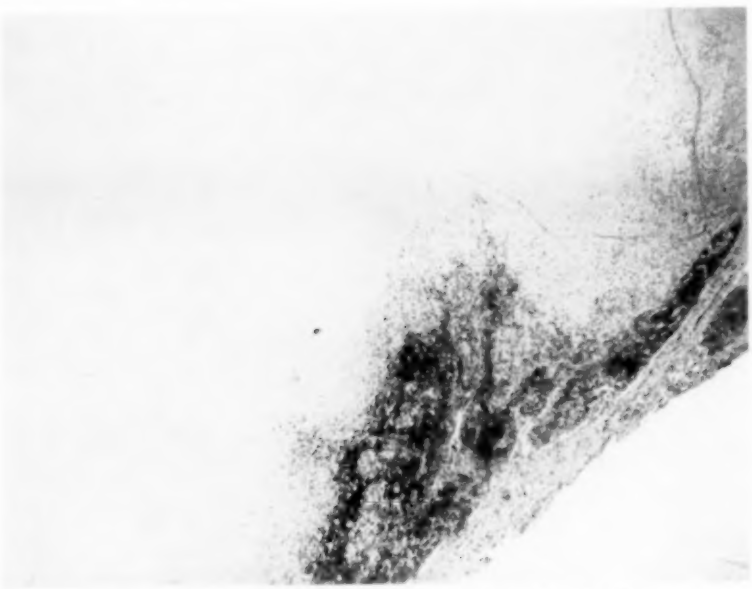

FIG. 2. Lymph node. In this section only a small portion of lymphoid tissue is present. The remainder of the specimen is made up of confluent masses of caseation necrosis. (×30)

and were arranged in ill defined cords. The cytoplasm was granular and eosinophilic staining. The nuclei were round to oval in shape and basophilic staining. Nucleoli were prominent (figure 5). The periportal areas revealed no increase in fibrous connective tissue, but there was slight infiltration by lymphocytes and mononuclear cells. Foci of lymphocytes and polymorphonuclear leukocytes were seen within the parenchyma and in the sinusoids. There was no evidence of hyalin eosinophilic cells or lipochrome laden Kupffer's cells (figure 6).

Course in the hospital. Because of the clinical evidence of toxicity and the initial abnormal urine findings, the patient was given 1.5 gm. of aureomycin daily for twelve days, a total of 18 gm. There was prompt clinical improvement characterized by subsidence of fever, malaise, nausea, vomiting, diarrhea, and abdominal distress. The lymphadenopathy noticeably regressed and continued so throughout the remainder of hospitalization. Because

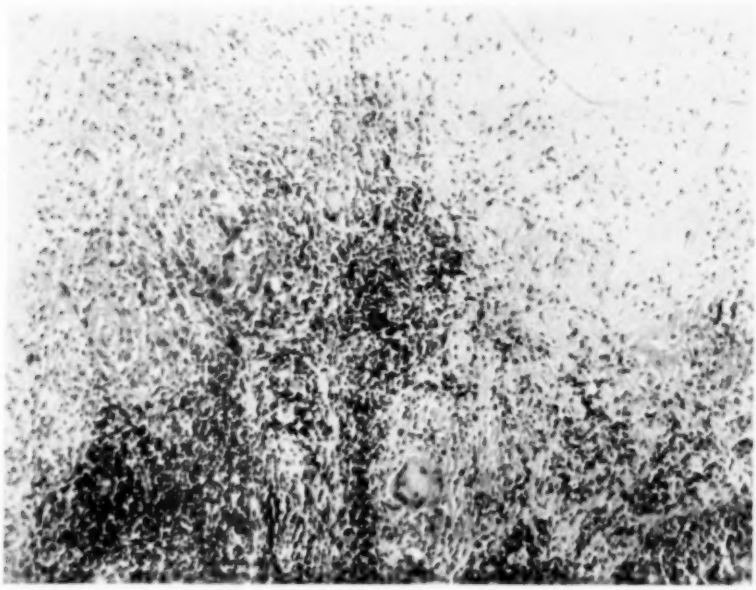

FIG. 3. Lymph node. At this power giant cells are seen at the edge of the granuloma. (×105)

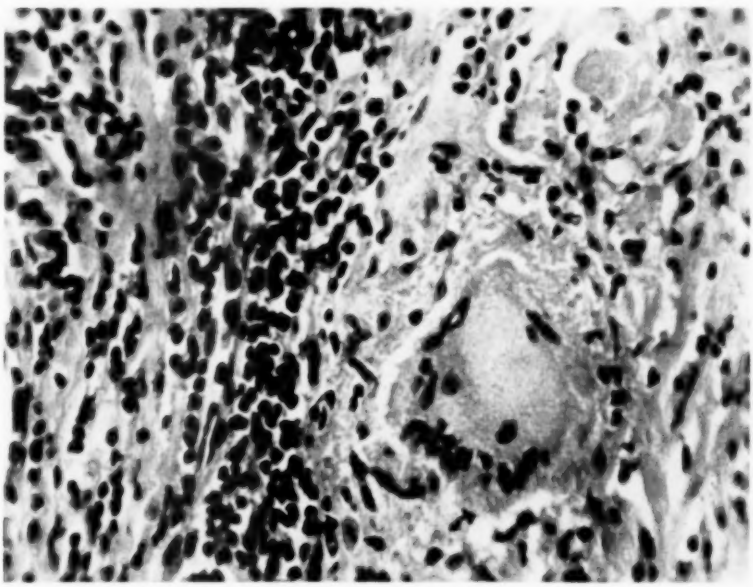

FIG. 4. Lymph node. Langhans' type giant cell surrounded by epithelioid cells. The adjacent rim of lymphocytes may be seen at the periphery. (×475)

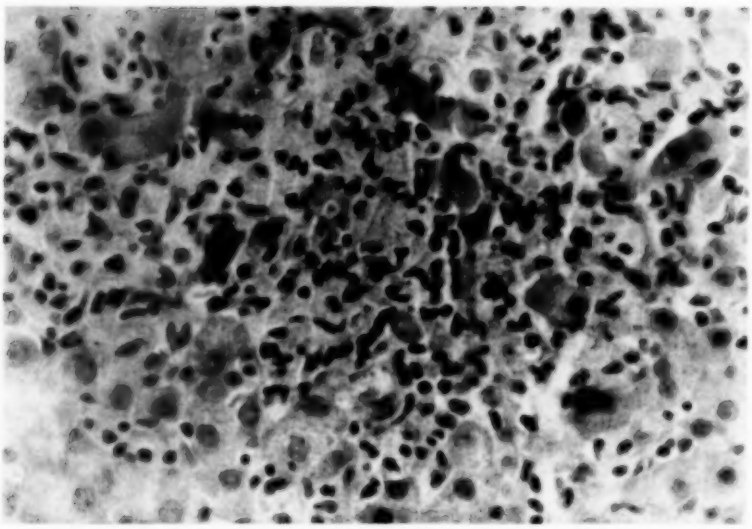

FIG. 5. Liver. The characteristic liver cord and ray pattern has been disrupted. There is a dense inflammatory infiltrate consisting of lymphocytes and polymorphonuclear leukocytes. (×475)

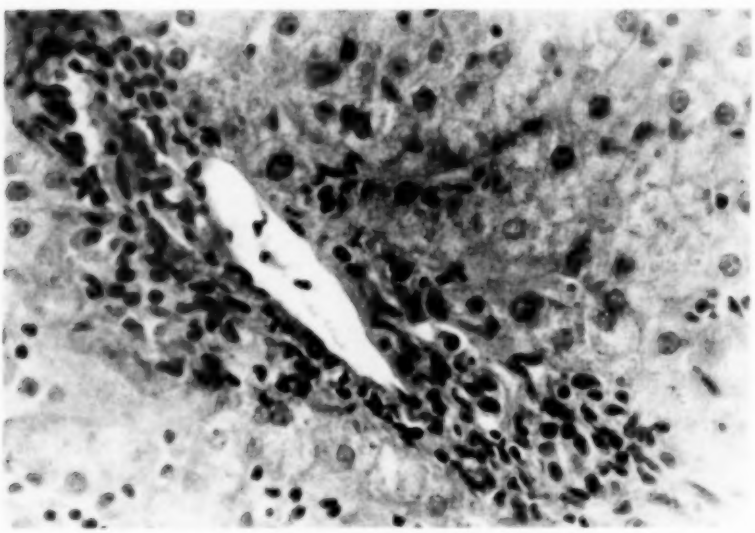

FIG. 6. Liver. The portal area reveals infiltration by mononuclear cells, the majority of which are monocytes. Note the disruption of the cords of liver cells in the periportal area. (×475)

of an initial abnormal agglutination for brucellosis, serial agglutinations and repeated blood cultures were obtained. The gradually rising *Brucella* agglutination titer and the growth of *Brucella suis* in blood cultures obtained five weeks after the onset of acute illness are revealed in figure 1. With these findings, a second course of aureomycin was instituted consisting of 0.5 gm. every six hours for fourteen days, a total of 28 gm. Blood cultures became negative following this therapy, and there was a gradual fall of the agglutination titer. In the latter half of hospitalization, due largely to improvement of appetite, the patient regained 10 pounds in weight. On discharge from the hospital he was asymptomatic, afebrile, and his general sense of well-being had returned to normal. It will be noted from figure 1 that two of the blood cultures obtained after return from a convalescent furlough were positive for *Brucella suis*. These reports were not available at the time of discharge from the hospital. The patient has been recalled for further therapy.

A search for similar cases was made in the records of the Armed Forces Institute of Pathology, Washington, D. C. Protocols of the autopsy and surgical specimens of all cases of proved or suspected brucellosis listed by the Institute were examined and included in the study. The protocols were especially scrutinized for descriptions of lesions which might contain caseous necrosis. Microscopic sections from all such cases were examined.

Although the material studied was obtained from 45 cases, only 10 of the group were proved bacteriologically. Clinical, laboratory, and historical details were insufficient in many of the cases for any definite conclusions. In general, it may be said that the histopathologic changes were not specific for brucellosis but were characteristic of a so-called chronic granulomatous inflammatory response. In none of the surveyed cases was there discovered a coexistence of caseating necrosis in the pathologic material and bacteriologic proof of *Brucella* infection.

DISCUSSION

The histopathology of brucellosis has been defined on the basis of changes observed in the tissues of experimentally infected animals and the tissues of patients who, having had the disease, died from brucellosis or other causes. The latter group has not been large due to the very nature of the disease and the possible lack of recognition of the condition during the patient's life.

In 1912, Fabyan (2) first called attention to the characteristic granulomatous lesion of brucellosis through his experimental studies on the guinea pig. Following intra-abdominal inoculation with known *Brucella abortus* strains, he observed lymphadenopathy in 95 per cent and splenomegaly in 98 per cent of his animals. Scattered over the surface of the spleen, liver, kidney, testes, and lungs were numerous fine, grayish, pinpoint elevations. His description of the microscopic changes is essentially the same as that presented today in standard pathology texts:

> It may be said that the essential changes associated with this disease are of a chronic inflammatory character and resemble those of tuberculosis to a startling degree, both in character and focal dissemination. The focal lesion consists of a group of epithelioid elements, i.e., cells with large vesicular usually oval nuclei, poor in chromatin. The cytoplasm stains very poorly and is ill defined. Among these cells may be a few nuclei with large peripheral blocks of chromatin and probably lymphoid cells. In some foci, plasma and giant cells may be noted. Occasionally polynuclear leucocytes are seen. Mitoses are numerous. Rarely focal necrosis is present. (2)

Throughout the literature, the role of the reticulo-endothelial system in the pathologic response to *Brucella* is stressed. It has been known for several years that *Brucella* tends to localize in organs containing abundant reticulo-endothelial tissue, namely, the bone marrow, liver, spleen, and lymph nodes. Similarly, the characteristic "granuloma" is featured by the presence of large mononuclear cells of reticulo-endothelial origin, often with associated peripheral lymphocytosis and monocytosis. This reaction is not specific for brucellosis because the same basic pattern is seen in such diverse conditions as tuberculosis, syphilis, the mycoses, lymphogranuloma venereum, sarcoidosis, Hodgkin's disease, and tularemia.

Chronic granulomatous inflammation, although studied extensively by many workers, remains something of a pathologic enigma. The morphologic characteristics, the source of its predominating cell, and the various injurious agents which produce this distinctive change are well known. The common stimulation, shared by each of the injurious agents, which sets into action this reticulo-endothelial response and eventually results in the familiar granuloma remains an unanswered problem.

The question of necrosis, complete or partial, in these granulomatous nodules of brucellosis frequently appears in reviewing the literature on the subject. It has been described in material from both infected animals and human cases (3-6). Braude (7) has demonstrated from his work with animals that the species of *Brucella* probably has a great deal to do with the response to infection, especially regarding abscess formation and necrosis. He has reported that *Brucella abortus* produced a disease characterized by little impairment in health, the presence of nonsuppurative granulomata, and marked splenomegaly. On the other hand, inoculation of the test animals with *Brucella melitensis* and *Brucella suis* produced widespread destructive abscesses demonstrating severe caseation. These same results have been obtained by many other investigators working from the experimental approach. Nevertheless, in 1947 Spink (8) wrote: "However, no description of caseous necrosis in the granulomas of brucellosis has been found." It can be assumed that he was referring to human infection.

At best, "caseation" is a poorly defined term. It stems from the Latin meaning "cheese," the mental concept of which entity best describes the gross features of the caseous lesion. It is generally accepted that most, if not all, body cells contain proteolytic enzymes which can act to liquefy the cell proteins after the death of the cell. While the primary function of these enzymes is concerned with the normal cell metabolism, their existence is also of considerable value in bringing about the liquefaction and removal of dead cells, so that replacement by new cells of the same type or repair by connective tissue formation may take place. However, as contrasted with the usual form of necrosis wherein complete autolysis takes place, the caseous lesion represents incomplete breakdown of the involved tissue; proteins and fatty acids exist or persist in abnormal amounts. Characteristically, the caseating lesion presents then necrotic cells which have lost their nuclei, structure, and outlines; their remains, together with intercellular materials, become fused into a formless coagulated cheesy mass.

Caseation in necrotic tissue is frequently mistakenly associated exclusively with lesions tuberculous in origin. Typical caseation, however, has been noted on repeated occasions in lesions produced by the typhoid bacillus, in syphilis and tularemia, in malignancy, in "lipoid pneumonia," in certain of the mycotic diseases, and in animal brucellosis. The prevalence of tuberculosis and the frequency of the caseous response to it undoubtedly accounts for this popular misconception concerning its so-called "specific" or "unique" lesion.

Throughout the years, investigative efforts toward elucidation of the true cause of caseating necrosis have continued. The studies have been varied and all have added to

our general fund of knowledge concerning inflammation. However, the basic cause of the caseating changes remains in doubt. Sabin (9), in 1941, reported on her study of the chemical fractions of the tubercle bacillus in an effort to trace their specific role in the pathogenesis of the infection. She reported that the tuberculo-phosphatid reproduced the characteristic tubercle and caseous necrosis. However, Rich (10) has pointed out that the relative quantities of that lipid fraction used were so enormous that the result falls entirely outside the realm of significance in naturally occurring disease. Whether the lipids contained within particular microorganisms have a role in caseation remains purely conjectural.

Another approach to the problem was made through the work of Jobling and Petersen (11), who demonstrated by *in vitro* studies that soaps of unsaturated fatty acids obtained from tubercle bacilli could inhibit the proteolytic activity of leukocytic enzymes. They suggested that such soaps might be responsible for the failure of proteolysis in caseous areas. Nevertheless, the problem still remains why this occurs in tissue killed by some bacteria and not in tissue killed by other bacteria.

Characteristically, caseation occurs in lesions wherein there is a predominance of mononuclear phagocytes. Weiss and Czarnetzky (12) have shown in rabbits that monocytes in that animal contain but one proteinase (pepsin) while the polymorphonuclear leukocyte contains three (pepsin, cathepsin, and trypsin). There was also a restriction of the range of activity of the monocytes as contrasted with the polymorphonuclear leukocytes relative to the pH of the medium. These studies are strongly suggestive for they are centered around the monocyte, the common factor shared by all caseating lesions, regardless of the inciting agent.

The patient of the present report presented the classical findings of acute brucellosis: recent exposure to a source of infection, generalized lymphadenopathy, fever, malaise, nausea, vomiting, diarrhea, weight loss, leukopenia, relative lymphocytosis, hepatic inflammation, rising titer of agglutinations for brucellosis, and positive blood cultures for *Brucella*. There can be no doubt of the diagnosis of brucellosis. The clinical response to therapy with aureomycin, particularly the regression of the lymphadenopathy, adds strong support to the belief that the lymphadenopathy was due to infection with *Brucella*. It seems quite likely that the caseation necrosis present in this case was also a manifestation of this infection. Recently the unusual coexistence of brucellosis and tuberculosis has been reported (13). In that case, the tubercle bacillus was obtained by culture of a bone marrow aspirate. In the present case, the caseation necrosis in the lymph node and the positive tuberculin test suggest the possibility of an associated tuberculous infection. It is believed, however, that the normal chest roentgenogram, the absence of acid-fast bacilli in the lymph node, and the lack of growth of tubercle bacilli in the culture of the bone marrow aspirate render this possibility unlikely. A review of the literature has failed to reveal any previous substantiated report of caseation necrosis in human brucellosis; the case at hand would seem to confirm the fact that this may occur.

The tendency for brucellosis to relapse despite present day antimicrobial therapy is also represented in this case. The initial course of aureomycin served to reduce the clinical symptoms to a minimum but did not eliminate the infection, as shown by the positive blood cultures within a month after cessation of therapy. The second course of aureomycin transiently cleared the blood stream of the organisms but again did not effect a "cure" of the disease. This course illustrates the chronic nature of the disease and the fact that infection may persist in the absence of positive clinical and serologic evidence of activity. In the management of brucellosis, emphasis should be placed upon careful and long-range follow-up observation with repeated courses of one or more antimicrobial drugs.

SUMMARY

A case of human brucellosis is presented with an unusual histopathologic response: caseation necrosis in a lymph node.

A review of the literature and of pathologic material from the Armed Forces Institute of Pathology indicates that the inflammatory reaction to the *Brucella* organism is, in general, nonspecific.

SUMARIO

Necrosis Caseosa en la Brucelosis Humana

Preséntase un caso de brucelosis humana con necrosis caseosa en un ganglio linfático, que constituye una extraña respuesta histopatológica.

Un repaso de la literatura y de ejemplares obtenidos del Instituto de Patología de las Fuerzas Armadas de los E.U.A. indica que la reacción inflamatoria al microbio *Brucella* es, en general, inespecífica.

RESUME

Nécrose caséeuse dans la brucellose humaine

Présentation d'un cas de brucellose humaine avec nécrose caséeuse d'un ganglion lymphatique, réaction histo-pathologique exceptionnelle.

Une analyse de la littérature et des travaux provenant de l'Institut de Pathologie de l'Armée indique, qu'en général, la réaction inflammatoire au microorganisme *Brucella* n'est pas spécifique.

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Editorial

PSYCHOLOGICAL ASPECTS OF TUBERCULOSIS[1]

Much has been written about the psychological aspects of tuberculosis within recent years. If one tries to separate the wheat from the chaff, the following facts emerge.

No uniform personality type prevails in tuberculous patients though a high degree of dependence and difficulties in handling hostilities have been found to be a common central feature in their premorbid personality. Previous to their illness some of these individuals display their inordinate need for maternal affection and allied dependent trends overtly, whereas others conceal them behind such psychological defenses as self-assertiveness, ostentatious independence, excessive self-drive, and aggressive and provocative behavior. Situations which entail a rebuff, a separation from, a threat of losing or an actual loss of a supporting figure often precede the onset of tuberculosis and its relapses.

These personality features may lead to a flare-up of a dormant lesion either by lowering of resistance owing to an unhealthy mode of life or by psychopathologic and autonomic mechanisms analogous to those operative in bronchial asthma. The person who "burns the candle at both ends" does so for reasons inherent in his personality.

Reactive phenomena may make themselves felt long before the illness has been diagnosed. Consciously or unconsciously patients suffering from tuberculosis respond to the *awareness* of initial symptoms by minimizing their importance or denying their existence. This denial mechanism is the prototype of many reactions which are encountered afterwards. For instance, to safeguard themselves against feelings of depression and of anxiety, which are the inevitable result of communicating the diagnosis, or because they cannot "afford" to be sick, many patients take recourse in disbelieving it, in minimizing the seriousness of their condition, or in refusing hospital admission.

Many phenomena which have been described as typical of tuberculous patients exist equally in other chronic diseases, e.g., inflation of self-interest, hysterical and hypochondriacal formations, overdependence, and a demanding need for affection.

Feelings of anxiety and of depression usually prevail in tuberculous patients. These feelings are normal and adequate responses so long as they are in keeping with the nature and degree of the anatomic lesion, and with the repercussions which the illness has in the patient's socio-economic situation. Discrepancies between subjective feelings and objective findings occur if the discovery of something bad inside himself is seized upon by the tuberculous patient's deep-seated feelings of guilt and is exploited as a confirmation of his belief in his badness. He

[1] Presented in part before the New York Tuberculosis and Health Association, New York, New York, on November 21, 1952.

may then express his self-condemnatory trends by regarding his illness as a well-deserved punishment. Conversely, the threat to the patient's life which actually exists may unconsciously be linked up with previous situations when misdemeanor on his part led to threats from his environment or from within himself. In this case neurotic anxiety is superimposed on justifiable fear. Analogous to manic patients, tuberculous patients may unconsciously deny their internal badness, i.e., they may adopt a foolishly defiant, ultracheerful, or overoptimistic attitude toward their illness (*spes phthisica*). Or they may eliminate their internal badness by projecting it onto the outer world. If this happens, they displace discontentment about their own condition onto the shortcomings of those whose duty it is to minister to their needs or onto the ostracism which in fact or in fantasy they experience.

Tuberculous patients have to subject themselves for prolonged periods to a state of childlike dependence on doctors and nurses and to a life of complete inactivity or at least of enforced idleness. No wonder that a substantial number of them surrender to their state of dependence while others endeavor to shake it off prematurely.

Hospitalization brings home to tuberculous patients that they are a potential menace and the necessity to accept dependency. Contact with fellow patients may act as a supporting factor or it may arouse so much anxiety—on various scores—that patients leave prematurely. Overlenient and overprotective management favors regressive trends and holds up recovery, whereas overseverity makes for foolhardy and obstreperous behavior.

Most patients feel anxious and uncertain of themselves on discharge from the sanatorium. Because they are afraid of the possibility of a relapse, some of them cling to their doctors and avoid the slightest exertion, sometimes in an absurd manner. Although apparently related to their illness, their anxiety arises from another source which requires exploring. Other patients at an early date, defiantly and against their own interests, keep away from their *doctors* because they want to forget their *illness*.

Other difficulties which await the homecomers arise from their reduced wage-earning capacity, from their lowered physical strength and vitality, and from their re-entry into the family circle and society in general. If the breadwinner happens to be the sufferer, lowering of standards and impairment of his prestige in the family structure can hardly be avoided. The ex-patient understandably blames himself for this state of affairs and is apt to suspect accusations where none exists. Members of the family may subject the homecomer to manifest or concealed fear of infection, may bother and irritate him by their oversolicitude, may seek their own amusements or even accuse the convalescent of overdoing caution. Some patients allow themselves to be dragged into strenuous activities rather than make themselves unpopular, not infrequently with disastrous results.

Persons who had previously been work-shy usually make the most of their disability and complain that excessive demands are made on them. Anxious individuals seek and content themselves with inferior jobs rather than endanger

their health. A depressive mood arising from thwarted ambition paralyzes a person's initiative and, like resentment, prevents him from making a wholehearted effort. Persons who deceive themselves about their handicaps or defiantly strive for more than they should do, not infrequently choose unsuitable occupations. Both attitudes may lead, not only to disappointment, but in some instances to a breakdown.

* * *

Some *practical conclusions* which were reached in conjunction with tuberculosis specialists may be permissible:

1. In the interest of both the patients and themselves, doctors communicating the diagnosis and its consequences are ill advised to raise unfounded hopes.

2. Should hospitalization be decided upon, the doctor—and nobody else—should induce the patient to accept it. Neither should the patient be frightened nor coaxed by blandishments into acceptance. Assistance by a social worker is an essential adjunct.

3. Harmony among the members of the therapeutic team is essential for the speedy recovery of the patients. Pivotal in this team is the *doctor* to whom, owing to their wish for recovery, patients are apt to attribute superhuman powers. A doctor who keeps his patients at arm's length fails in his duty of assuaging their anxieties regarding the illness, the situation of the family, and their own future. Whether or not to inform patients about their condition may be debatable. But by and large it is probably true that patients resent being treated as if they were not old enough to know what is the matter with them. Explanatory talks, broadcast or otherwise, on tuberculosis and the uses of the sanatorium, a feature "Any Questions?" and group discussions have been an outstanding success. Only in the case of patients who are irrevocably going downhill is adherence to the truth definitely out of place.

A *nurse* who is ignorant about her patients' physical condition is unable to handle them intelligently; whereas a nurse forced into detachment is deprived of her desire to give maternal care which, in their regressed state, her patients badly need. Conversely, a nurse who "mothers" her patients excessively may gratify her own emotional needs but does not necessarily serve the interests of her patients.

Primarily the function of the *social worker* is to assist the patients to adjust themselves to their social environment; hence, the designation. But she can carry out this function successfully only if she pays due regard to the patients' emotional state; otherwise she may land herself in the position of an almoner in its literal connotation.

4. In regard to the *patients*, as far as possible, individual requirements should be taken into account. Conscientious, self-driving individuals should be encouraged to do less than their severe social sense allows them, and anxious, self-centered individuals to do more than their fear of reverses and relapses lets them.

During the initial stage patients should be allowed to pass through a phase of depression without much interference, for after all they have good reason to feel depressed. Nobody can seriously believe that advice that "they should pull themselves together" or that "they should not worry" can influence their state of mind in any way. Similarly, anxious patients who frantically try to conceal their anxiety

should be tactfully encouraged to admit their fears without feelings of shame. Afterwards, with the proviso made previously, the aim all along should be to induce the patients to make a realistic assessment of their situation.

5. It is essential to bring home to patients the therapeutic value of rest and the fact that those on conservative treatment are not necessarily worse off than those who receive surgical treatment. Fear of operations is justified; if it assumes abnormal proportions, ridicule or annoyance do more harm than good. Misconceptions should be dispelled, while phobias may require expert attention. Failure of an operation will hardly ever impair a patient's trust in his doctor if he has been forewarned, or if an explanation for the failure has been given to him or, if necessary, to the ward afterward.

6. A fair hearing should be given to any grievance or grouses. In most cases a simple talk between the patient and the medical superintendent will settle all difficulties.

7. Persons who have always rebelled against authority, family-bound individuals, duty-bound individuals, and those who cannot tolerate communal life or life in a community composed of members of their own sex are potential "self-dischargers." Patients with minor lesions may quit because they fail to see the reason why they should stay; while patients who progressively go downhill despite sanatorium treatment may depart prematurely on the logical grounds that they do not get better anyway, and on the illogical grounds that removal from the scene of anxiety may lead to removal of the source of anxiety. Fear of operations given as a reason for signing out falls in the same category. No magic formula can be offered to deal with this problem, which is obviously only one segment of the total problem of sanatorium management.

8. A superintendent who runs his sanatorium on the lines of a remand home can hardly be surprised to have a good deal of trouble; on the other hand, reasonable liberalism and a laissez-faire laissez-aller attitude should not be confused.

Passive entertainments such as movie shows do not succeed in counteracting regressive trends. The same objection applies to some extent to traditional occupational therapy. As the patient emerges from his regressive dependent state he should be encouraged to embark on activities which require his active participation, such as education courses, directed reading, work at a patients' council and in committees.

9. Prior to discharge, a heart-to-heart talk of the medical superintendent or one of his senior medical officers with the patient and with members of his family seems essential; or, if this is impossible owing to lack of time, an after-care talk to a group of patients proves of great value. A trial period at home some weeks prior to the final discharge from the sanatorium may be helpful.

10. In the past it has been a common practice to give vocational guidance to tuberculous patients, mostly on the basis of their physical condition and on the basis of what they wish to do. However, presence or absence of drive on the part of the patient, fear of doing too much or desire to do more than is wise, hysterical exploitation of, and compulsive urge to disregard, handicaps are all factors which should also be taken into account.

The view is generally accepted that tuberculous workers cannot easily be absorbed by normal industry. The solution of the problem seems to be sheltered employment. However, sheltered employment—hence, its designation—entails

continuation of protection in the work environment. Such protection may be necessary but should not be perpetuated beyond the limits imposed by the patients' condition because it reinforces their dependent trends.

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Notes

VIABILITY OF TUBERCLE BACILLI IN VIVO WITH AND WITHOUT CHEMOTHERAPY

At the request of the Editor, Dr. Dubos has kindly consented to incorporate the substance of his remarks at the Conference on Chemotherapy of Tuberculosis of the Veterans Administration, held in February, 1953, in the form of the present communication which is being presented here through the courtesy of Dr. Arthur M. Walker. The full text will be published in the Transactions of the Twelfth Veterans-Army-Navy Conference on the Chemotherapy of Tuberculosis, February 9–12, 1953.

The papers which have been presented before this conference illustrate two types of phenomena which appear at first sight contradictory, and indeed incompatible. On the one hand, the fact is now clear that, despite the availability of several types of drugs highly effective against tubercle bacilli *in vivo*, it is very difficult, if not impossible in most cases, to eradicate infection from human patients, even by prolonged chemotherapy. In contrast is the other fact, long known to pathologists, that bacilli tend to disappear spontaneously and without the benefit of any form of therapy from certain types of lesions, in particular from cold abcesses and from closed caseous areas. This is well confirmed by the observation reported today that it is at times difficult to recover living bacilli from resected lung lesions in patients who have received no chemotherapy whatever. Instead of being contradictory, however, these two phenomena correspond in reality to two independent aspects of the pathogenesis of tuberculosis. I shall devote much of my discussion to the presentation of some experimental findings bearing on this problem.

The failure of drugs to kill all bacilli in infected tissues has been observed, not only in human tuberculosis, but also in many types of experimental infections. I shall illustrate the findings with examples taken from mouse tuberculosis; in part, because I have had some personal experience with this type of disease and, even more, because it has been my privilege to discuss its course on several occasions with Drs. McDermott, Tompsett, and McCune, of the Department of Medicine of the New York Hospital. These investigators have kindly permitted me to quote to you some of the facts discovered by Dr. McCune while he was studying quantitatively the effect of various chemotherapeutic agents on the multiplication and survival of virulent tubercle bacilli in mice.

I shall attempt to condense very briefly results obtained independently by Dr. McCune and in our own laboratory. Mice were infected by the intravenous route with various doses of culture containing approximately from one hundred to one million organisms. Several strains were used, some virulent (H37Rv); others attenuated (R_1Rv and BCG). The animals were treated with either streptomycin or isoniazid, or simultaneously with both drugs, the therapy being started on the very first day of infection and continued thereafter. Some of the animals were sacrificed at weekly intervals over a period of three months. Although no macroscopic evidence of tuberculous lesions could be recognized at any time in any of the animals, living tubercle bacilli could be recovered from all of them, even when BCG was used and when therapy had been continued for eighty-five days. Moreover, bacillary invasion of the tissues began uniformly within a few days after discontinuance of the drugs. Several lines of experimentation have thrown some light on the mechanism of this disturbing phenomenon.

First, there must be recognized the simple but all important fact that, despite early claims, it is practically impossible to sterilize cultures of tubercle bacilli *in vitro* by adding to them either streptomycin or isoniazid, even in high concentrations. True enough, most of the bacilli appear to die rapidly in contact with the drugs, but a few survive prolonged exposure to them, as recently shown by H. Noufflard (Ann. Inst. Pasteur, 1953) and by Dr. R. Tompsett (personal communication). Whether these survivors correspond to unrecognized stages in a life cycle of the bacilli or merely to forms in a state of metabolic quiescence has not yet been established. But it is of extreme importance from our point of view that the phenomenon probably has its counterpart *in vivo*. It is probable, in other words, that a certain percentage of the bacilli in an infected individual survive exposure to the drugs, not because they have developed hereditary "drug resistance" in the sense so widely studied during recent years, but because they exist in a form different from that in which observations are made under ordinary circumstances.

Another reason for the difficulties experienced in eradicating infection may be found in the fact that *in vivo* a large percentage of the tubercle bacilli are present, not free in body fluids, but instead are within phagocytic cells. Experiments in tissue cultures have revealed that the inhibitory power of streptomycin fails to manifest itself against the growth of bacilli which have been engulfed by monocytes—a fact which certainly contributes to the inability of this drug to eradicate infection (Mackaness, Suter).

This explanation, however, cannot serve in the case of isoniazid, for this drug is just as effective intracellularly as it is extracellularly, in tissue cultures at least. It must be assumed, therefore, either that the bacilli can be engulfed by cells which behave toward the hydrazide in a manner different from that of the monocytes or, more likely, that some factor in the *in vivo* environment antagonizes antibacterial activity. In this respect, it may be worth mentioning that isoniazid can be rendered inactive *in vitro* by adding to the culture medium the proper concentration of sodium pyruvate, a normal metabolite (W. B. Schaefer, personal communication). In fact, the problem of the existence in tuberculous lesions of substances capable of inhibiting antimicrobial drugs is one worthy of more attention than it has received heretofore. Areas of necrosis, particularly of caseation necrosis, contain a variety of partially broken-down tissue components of unknown chemical composition, many of which are likely to act as drug inhibitors. Moreover, the reaction of these areas is often acidic and, hence, unfavorable to streptomycin. The finding that drugs penetrate caseous matter—as has been reported in the past and well demonstrated again this afternoon—is no warrant, therefore, that they can exert their antibacterial action in the physicochemical environment of the lesion.

I shall now consider the fact that bacilli often disappear spontaneously from certain types of lesions, without the help of chemotherapy. This fact is so well documented, for example, in Canetti's recent book, that there is no need to give more evidence of it. As mentioned in the discussion which I presented last year before the Eleventh Conference, my colleague, Dr. J. Hirsh, and I have recognized the presence in animal tissues of several substances which might be responsible for this effect. These substances are:

(*a*) The naturally occuring amines, spermine and spermidine, which become inhibitory to tubercle bacilli after being modified by the tissue enzyme spermine oxidase.

(*b*) The enzyme lysozyme which does not lyse the bacilli, but can kill them rapidly even when tested in highly dilute solution (1:100,000).

(*c*) A basic polypeptide which we have recently isolated from the thymus gland.

I pointed out also last year that organic acids accumulate in and around tuberculous lesions as a result of the anaerobic metabolism of inflammatory cells and of the activity

of the lipolytic enzymes released during necrosis. Recent experiments have shown that bacilli become unable to multiply in ordinary media, and fail to produce infection in laboratory animals, after having been exposed for several weeks to physiologic concentration of the sodium salts of several organic acids. Sterilization of the cultures occurs in particular when the atmosphere is completely or partially anaerobic and when the CO_2 tension is higher than normal. I need not point out that these are precisely the conditions prevailing in certain types of closed lesions.

It may be proper to consider briefly at this time a problem which has loomed very large in your discussions during the past two years, namely, whether the bacillary forms which can be seen in resected lesions but which fail to grow in culture media and to cause disease in experimental animals should be regarded as "dead," or merely as dormant but potentially viable, bacilli. I cannot answer this question; moreover, I shall make bold to say that none of the evidence adduced so far is of much help in answering it. In my opinion, the techniques used by the various investigators were not adequate to determine the potential viability of dormant bacillary forms. The problem is so complex that I can do no more than present a few selected facts to illustrate its many facets. Some experimenters claim that reduction of tetrazolium or of some other dye, uptake of oxygen or CO_2 production, and other metabolic tests can be used as an index of viability of the bacilli. In reality, however, many procedures and many chemicals are known which render microorganisms unable to multiply and yet allow them to continue to respire and metabolize. An analogy will make this point clear. Animals which have been castrated are sterilized, unable to reproduce themselves, yet they can still respire, metabolize, and grow. Should they be considered dead or living? The tetrazolium test, so much in fashion these days, measures only the ability to reduce and this ability can be retained by cells which are no longer capable of multiplying. All metabolic tests suffer from the same limitations.

In contrast, organisms which respire or reduce only poorly may merely have exhausted some essential metabolite, or be in a resting form akin to that of spore, and yet may be able to multiply when placed in the proper environment. It is possible, furthermore, for a cell to adsorb from the environment some inhibitor which prevents its multiplication until circumstances are provided for the removal or neutralization of the inhibitor. Examples of this type of apparent death followed by revival under the proper conditions are common in many fields of microbiology and cannot be reviewed here. Until now, only standard media and standard inoculation and infection techniques have been used to test the viability of tubercle bacilli present in pathologic materials. These procedures are probably not adequate to determine the viability of organisms which are present in a dormant, latent state. One needs only to recall in this connection the variety of "latent" infections caused by protozoa, bacteria and viruses, in which the causative agent can be brought out only by very special "evoking" techniques.

Finally, one should not overlook the fact that tubercle bacilli are present not only in the parenchymatous lesions but also in lymphatic tissues. In cattle which have received a virulent infection after being vaccinated with BCG and do not show any overt sign of tuberculosis, the virulent bacilli can be found without fail in various lymph nodes for months and years thereafter. It is known that even BCG bacilli can persist for several years in the lymph nodes of man and animal. And experiments in our laboratory have revealed, furthermore, that prolonged therapy with isoniazid fails to eradicate BCG infection.

All these facts leave one with the impression that no technique is as yet available to bring about with certainty a complete eradication of the bacilli from infected tissue.

After antimicrobial drugs have ceased to exert their restraining influence on infection, either because the infective organisms have become resistant to them or because therapy has been interrupted, only the resistance of the host can act as a break on reactivation of disease caused by the bacilli surviving here and there, in one form or another, detectable or not by the classical methods of pathology and bacteriology.

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February 10, 1953.

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ISOLATION OF AIR-BORNE TUBERCLE BACILLI IN A TUBERCULOSIS HOSPITAL

For more than sixty-five years reports have indicated that respiratory tuberculosis may be naturally acquired by animals when placed in a room where tubercle bacilli are being released by tuberculous animals.[1-4] Augustine[5] reported samples of dust containing virulent tubercle bacilli in 25 per cent of the rooms sampled where patients were confined with pulmonary tuberculosis; this is considerably higher than was demonstrated from samples of air where one guinea pig became infected with tuberculosis from 25 samples of air.

Lurie,[4] reports that ultraviolet lights are effective in killing tubercle bacilli under certain experimental conditions. Lurie[6] also indicated that ultraviolet irradiation of the air exercises a protective influence against natural air-borne contagion of tuberculosis in the rabbit, where the tubercle bacilli are air-borne from the urine of infected animals.

Due to these past indications ultraviolet lights were installed in the laboratory and animal house at Battey State Tuberculosis Hospital.

The study reported in this paper gives a procedure by which tubercle bacilli were isolated from the air in the laboratories previous to the installation of ultraviolet lights. The initial aim of the project was to determine if a reduction in viable tubercle bacilli could be demonstrated after ultraviolet light installation. Sufficient tubercle bacilli isolations were not made in the initial phase to continue the reduction study on a statistically significant basis after ultraviolet installation. Air was sampled from several strategic areas in the hospital, namely, the sputum preparation and gastric collection rooms in the laboratory, and the operating room where bronchoscopic examinations were performed.

Several air sampling devices and methods were employed with only one, the Greenburg-Smith type of impinger,[7] giving an isolation of tubercle bacilli. This instrument was, therefore, made the apparatus of choice for this study. The "G. E. Electrostatic Air-Sampler"[8] was very satisfactory in total bacterial reduction studies,[9] but unsatisfactory in regard to tubercle bacilli isolation regardless of the medium or methods employed.

The Greenburg-Smith type of impinger concentrator was connected to a vacuum pump through a flowmeter. The air was drawn through 100 ml. of sterile distilled water at a rate of 1 cubic foot per minute. In the gastric collection room, approximately 60 cubic feet of air were collected during each sampling day; and, in the sputum preparation room, approximately 480 cubic feet of air. Samples were taken for twenty-five days in each room, and all collections were made only while routine work was in progress. After

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the pump was cut off, the intake tube was washed down with sterile distilled water. The suspensions were poured into sterile centrifuge tubes and centrifugalized at 3,000 r.p.m. for fifteen minutes. The supernatant fluid was discarded, and the sediment was treated with 4 per cent NaOH for ten minutes, then neutralized with HCl in the presence of phenol red as an indicator. One half of the sediment was planted on four tubes of Jensen's modification of Löwenstein's egg medium,[10, 11] and the other half was injected subcutaneously into the groin of a tuberculin-negative guinea pig.

Five samples of air for tubercle bacilli were collected from the operating room where bronchoscopic examinations were performed. The procedure given above was modified after centrifugalization. The supernatant from these samples was not discarded but injected, untreated, subcutaneously into the groin of tuberculin-negative guinea pigs. No cultures were planted. Using this procedure one would not discard any microorganisms which might possibly be in the supernatant, and the microorganisms are not subjected to any chemical which might render them nonviable.

TABLE 1

AIR SAMPLES FOR THE DETECTION OF TUBERCLE BACILLI

ROOMS	NUMBER OF AIR SAMPLES	NUMBER OF SAMPLES CONTAINING ACID-FAST ORGANISMS			GUINEA PIG RESULTS POS.*	TOTAL NUMBER SAMPLES POS.*
		Microscopic	Culture			
			Saprophytes	Pos.*		
Gastric collection	25	1	7	1	1	1
Sputum preparation	25	1	8	1	1	1
Bronchoscopy	5	2†	—	—	1	1
Total	55	4	15	2	3	3

* Positive for *M. tuberculosis*.

† Laboratory accident prevented injection of one which was positive microscopically.

All cultures in this study were examined periodically for acid-fast bacilli, and final readings were made at ten weeks. All animals were autopsied at approximately ten weeks after injection.

All satisfactory air samples for the detection of tubercle bacilli were collected with the "Greenburg-Smith" type impinger, which has been used extensively in the determination of industrial dust.

From the data in table 1 it will be noted that, of the microscopic examinations of 50 smears from the sediment of the air samples taken from the gastric collection and sputum preparation rooms, acid-fast bacilli were demonstrated in 2 instances (one from each room). The guinea pig injected with the sediment of one of these positive samples gave a weakly positive tuberculin test, but on autopsy no gross evidence of tuberculosis was found. The other animal was negative to tuberculin and revealed no evidence of tuberculosis at autopsy.

On culture of the 25 samples from the gastric room, 8 produced acid-fast bacilli. One of the 8 acid-fast cultures gave a typical human-type colony and was shown by animal injection to be *M. tuberculosis*. Of the 25 samples from the sputum room, 9 produced

[10] HOLM, J., AND LESTER, V.: Acta tubere. Scandinav., 1941, *16*, 3.

[11] JENSEN, K. A.: Zentralbl. f. Bakt. (abt. 1), 1932, *125*, 222.

acid-fast bacilli. One of the 9 acid-fast cultures was of doubtful identity, and injection of the sediment from the original sample into the guinea pig produced an infection only in the superficial inguinal lymph node at the site of injection. An injection of the questionable culture (1 ml. of a 10^{-1} dilution) subcutaneously into the groin of a guinea pig produced an infection in the superficial inguinal, deep inguinal, and common iliac nodes with purulent exudate which contained acid-fast bacilli.

The same apparatus was used on 5 separate occasions in the operating room during bronchoscopic examinations. Acid-fast bacilli were revealed on microscopic examination of 2 of the 5 samples collected. A laboratory accident prevented the injection of one of the 2 samples, but both the untreated supernatant and the sediment of the other produced tuberculosis in the guinea pigs.

It should be understood that the air was drawn through sterile distilled water at the rate of one cubic foot per minute. With this method, many fine particles of the size of isolated tubercle bacilli may pass through the water and not be trapped thereby. No examination was made of the effluent air from the Greenway-Smith impinger to determine if it was free from tubercle bacilli. Conceivably, the foregoing data may have under-rated the full magnitude of air contamination.

In summary: A procedure using the Greenburg-Smith type of impinger with distilled water is given for the isolation of tubercle bacilli from air when the concentration is extremely small. Of 50 air samples collected in two laboratory rooms, 2 samples contained tubercle bacilli. Of 5 air samples from one operating room, one sample definitely contained *M. tuberculosis*, and one was probably positive but was not inoculated into a guinea pig because of a laboratory accident. Acid-fast saprophytes were common in the air of this laboratory.

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ROME, GEORGIA
GEORGIA DEPARTMENT OF PUBLIC HEALTH
ATLANTA, GEORGIA
FEBRUARY 26, 1953

H. MAC VANDIVIERE[12]
C. EDWIN SMITH
E. J. SUNKES

[12] Present address: North Carolina Sanatorium, McCain, North Carolina.

Books

TUBERCULOSIS NURSING INSTRUCTION IN UNIVERSITIES FOR PUBLIC HEALTH NURSING STUDENTS. *By Jean South.* Pp. 28, *Joint Tuberculosis Nursing Advisory Service, New York,* 1952.

This study was made to determine the tuberculosis nursing content and field experience offered in programs of study approved for public health nursing by the National Nursing Accrediting Service. Twenty-eight of the 34 universities returned questionnaires and gave information about the content in tuberculosis received by 267 students who were having field experience in 51 field agencies during the fall or winter term of 1950–1951.

Universities preparing students for public health nursing positions will be especially interested in this report, and it should be read by persons planning similar studies of other aspects of public health nursing programs. The difficulties in obtaining comparable data from universities with such diverse programs are recognized but the apparent discrepancies in the report do limit the value of the study. Considerable variation was found in the tuberculosis content of the programs studied but some of the variation might be due to the interpretation of the questionnaires. Since a range of 6 to 640 hours for field work is reported, it would seem that some universities may have reported hours of university credit received while others reported actual clock hours spent in the field. There is some confusion in the use of such words as "credit," "term," and "session."

Some of the material in the report is presented in such a form that it is difficult to interpret without referring to *The Public Health Nursing Curriculum Guide.* The report might be more useful if the reader had been referred to the questionnaire in the appendix of the report. The form in which table 3 is presented is also rather confusing.

The recommendations in the report appear to be sound, but they are not always supported by the data in this study. The report states that there is considerable variation in the amount of tuberculosis content in these programs; that many students are not being prepared to meet the needs of service programs; and that one-quarter of the universities are not giving sufficient instruction in the "Medical Aspects of Tuberculosis" or in the "Tuberculosis Control Program." The criteria for the evaluation of tuberculosis content in public health nursing programs do not seem adequate to justify these statements, especially when no consideration is given to the experience which students have had before entering the university.

The suggestion that universities require that deficiencies in tuberculosis be made up before the student enrolls in the university or made up along with the other requirements of the university seems sound but would need to be studied in relation to total university and agency policies. Many public health agencies are still sending students to school for only two semesters, one of which is devoted to field work. Whether students should be permitted to study public health nursing before making up basic nursing deficiencies is a very real question but does not seem to be within the scope of this study.

Several of the suggestions for improving the content in tuberculosis imply that universities are not carrying out the recommended procedures, although the questionnaires did not ask for this information. The report suggests that universities appraise the student's knowledge of tuberculosis upon admission to the school; that universities should

know whether or not field agencies include tuberculosis nursing services; that agencies and universities should work together to coordinate learning experiences; and that universities learn from directors of nursing how competent their graduates are in tuberculosis nursing. It would be unfortunate if readers assumed that the universities reported that they are not doing these things.

Although this review seems to emphasize the limitations of the study, the experience gained in trying to evaluate the content of one area of public health nursing should be of real value. In another study some of the pitfalls encountered here could be avoided. This study shows the need for a clear definition of terms to be used and for establishing criteria for evaluating the program. *The Public Health Nursing Curriculum Guide*, published in 1942, may not be adequate for this purpose.

Ella E. McNeil
Ann Arbor, Michigan

BOOKS RECEIVED

Tuberculosis in the Commonwealth. Transactions of Third Commonwealth Conference. Pp. 443, with illustrations, National Association for the Prevention of Tuberculosis, Tavistock House North, London, 1953, Price, one guinea.

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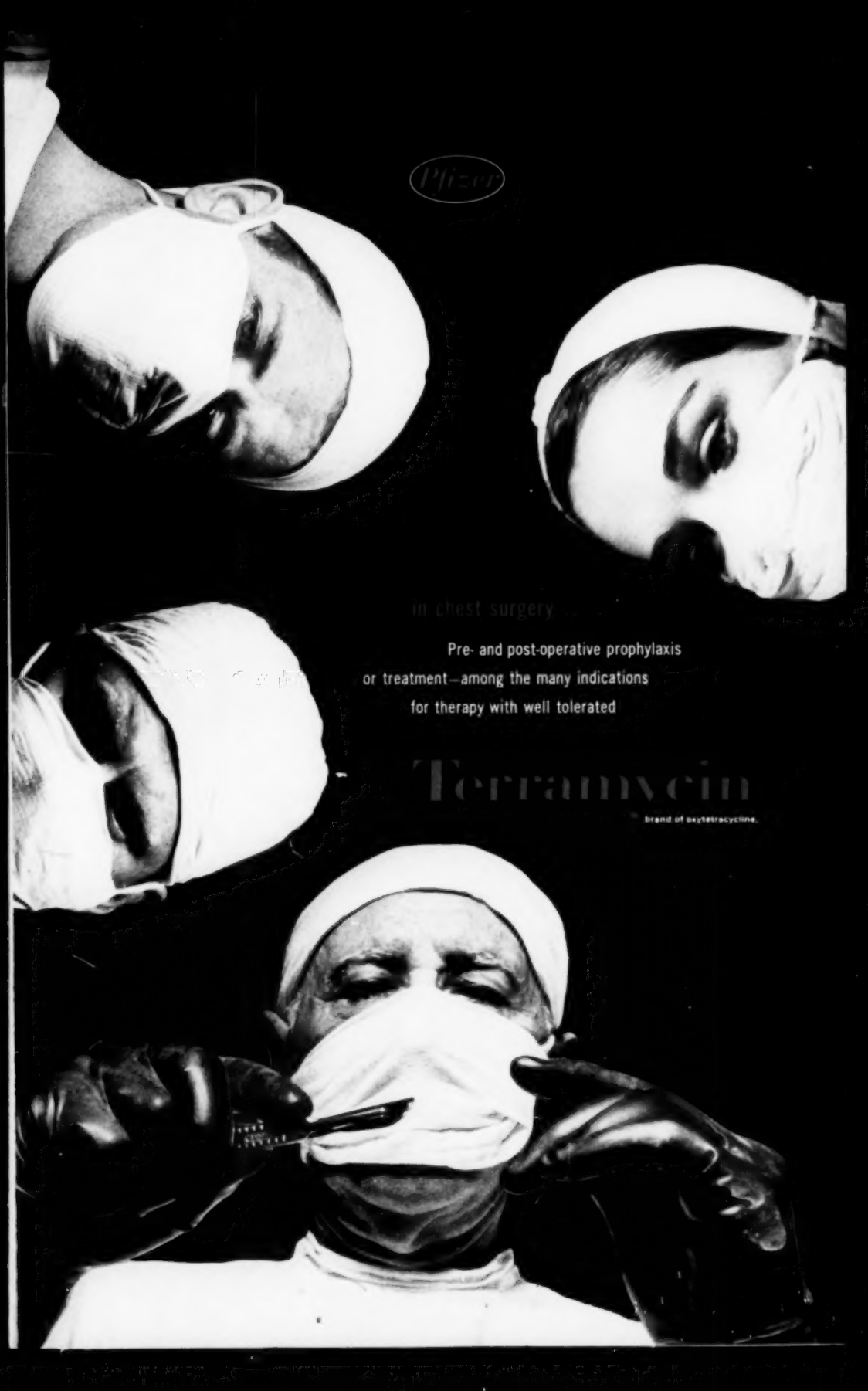
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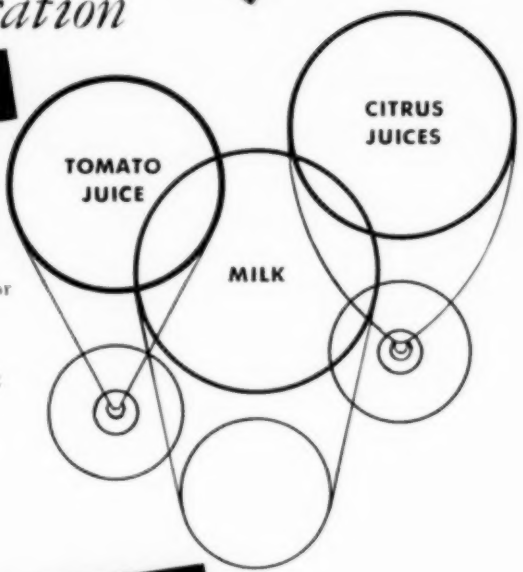

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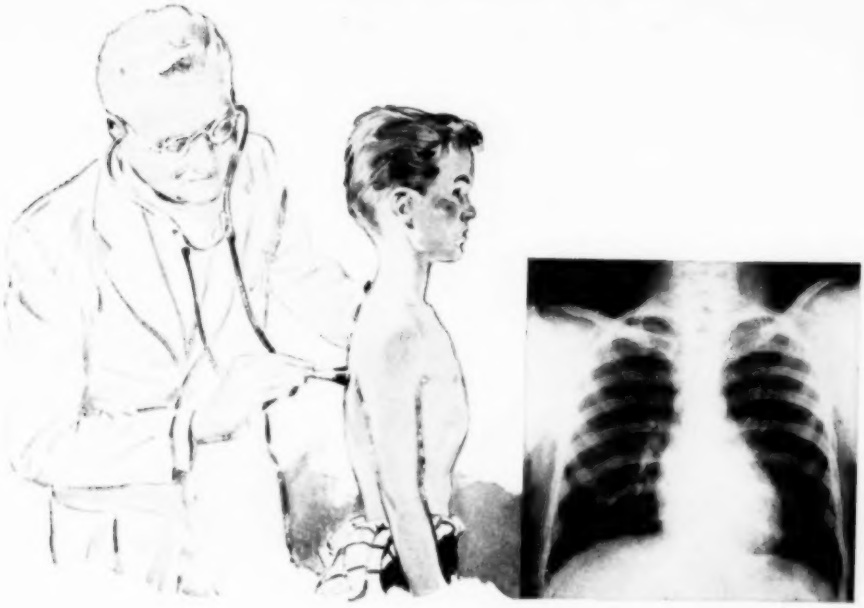

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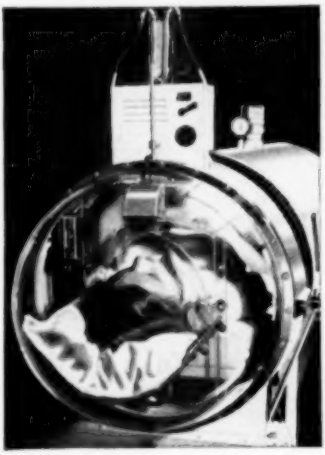

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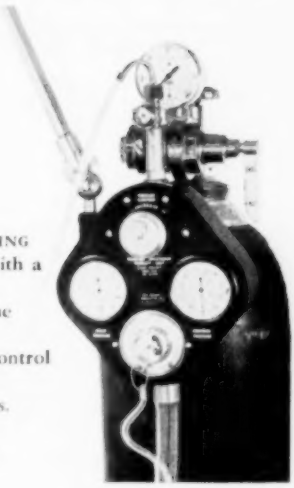

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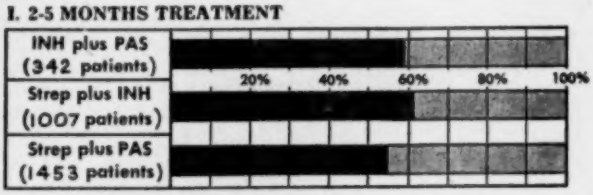

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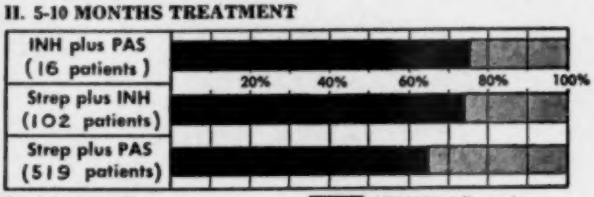

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